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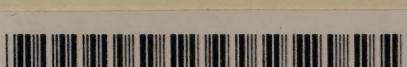

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TRANSACTIONS
OF THE BRITISH INSTITUTE
OF PREVENTIVE MEDICINE

Transactions of the British Institute of Preventive Medicine

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EDITORIAL NOTE.

THE papers included in this volume have been contributed by members of the Staff of the Institute, and were completed early in the present year.

ALLAN MACFADYEN,
Director.

October, 1897.

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INTRODUCTION.

THE best introduction to this volume of papers will be a short account of the work of the Institute it represents.

The British Institute of Preventive Medicine was incorporated on July 25th, 1891, with the view of founding in the United Kingdom an institute similar in character and purpose to the "Institut Pasteur" in Paris, the "Hygienisches Institut" in Berlin, and other establishments of a like nature existing abroad.

The main objects of the Institute, as set forth in the Memorandum of Association, are as follows:—

(1) To investigate the means of preventing and curing the various infective diseases of men and animals, and to provide a place where researches may be carried on for this purpose.

(2) To provide instruction in preventive medicine to medical officers of health, medical practitioners, veterinary surgeons, and advanced students.

(3) To prepare and to supply to those requiring them such special, protective, and curative materials as have already been found or shall in future be found of value.

Further, to provide the means for carrying out investigations in all branches of Bacteriology, including those of practical importance to chemists, agriculturists, and manufacturers.

No provision previously existed in this country for the promotion of these objects on a scale at all commensurate with their importance.

A Council was appointed to govern the affairs of the newly founded Institute, representing in its members biological, chemical, medical, veterinary, and agricultural science.

A public appeal was made for the necessary funds, and the response showed that the idea of a National Institution for the study of Bacteriology met with wide approval. The Council were thus enabled to commence the work outlined in the statutes of the Institute, viz., "to provide laboratories, to appoint a scientific staff, to institute lectures and demonstrations, to issue publications of the transactions of the Institute, and to found a library."

The College of State Medicine was amalgamated with the Institute in 1893, and in this way temporary premises were obtained at Great Russell Street. A scientific staff was appointed, and provision was made for research work, lectures and demonstrations.

In the meanwhile a site was purchased at Chelsea, from His Grace the Duke of Westminster, and the building of a permanent home for the Institute commenced.

The development of serum-therapeutics led to the establishment of an antitoxin department at Sudbury, and to the preparation of the diphtheria serum for the first time in this country. A number of donations were received for the purpose of inaugurating this work and expanding its scope. The preparation of the tetanus antitoxin, the anti-streptococcic and the anti-pneumococcic serum, was also undertaken, in addition to researches in the field of serum-therapeutics.

The bacteriological and chemical departments at Great Russell Street were utilised for investigation and instruction. A number of original papers have already been contributed to various journals, whilst the laboratories have been attended by workers from all parts of the world.

The trustees of the late Mr. Richard Berridge granted a sum of money to the Institute for the purpose of building and endowing a water laboratory, where researches on the bacteriology and chemistry of water and sewage could be conducted. A chemist was appointed to take charge of this important branch of work, and a number of researches have been carried out, some of which appear in this volume.

The following papers have been contributed by members

of the staff of the Institute, and are published for the first time.

It is anticipated that the new building at Chelsea will be ready for occupation early next year, when adequate accommodation and proper facilities will be provided for the full organisation of the work of the Institute. The Institute may then feel justified in issuing a still fuller volume of Transactions.

LISTER.
Chairman of Council.

TRANSACTIONS

OF THE

BRITISH INSTITUTE OF PREVENTIVE MEDICINE.

A CONTRIBUTION TO THE STUDY OF STREPTOCOCCUS PYOGENES.

BY WILLIAM BULLOCH, M.D.

THE question of the identity of streptococcus erysipelatis (Fehleisen) with streptococcus pyogenes (Rosenbach) has been much debated. Petruschky[1] however, has recently demonstrated that a typical erysipelas can be set up in the human subject by the inoculation of living cultures of a streptococcus obtained in purity from a case of suppurative peritonitis. It seemed to be a point of interest as a further proof of identity to determine to what extent an animal, immunised against a given streptococcus, would react to cultures of streptococci obtained from entirely different sources. The experiments detailed below have reference mainly to this point.

As is well known, streptococci exhibit very great variations as regards their pathogenic action on animals. A transient redness at the point of inoculation may be all that is seen. At other times there may be a severe erysipelas, or a phlegmon. The animal may die with a large local lesion or death may ensue from a rapidly fatal septicæmia, while locally nothing may be found, or at most a slight gelatinous-like œdema. It is also known that streptococci

[1] Petruschky *Zeit. f. Hygiene*, Bd. xxiii., Hf. i., p. 142.

from severe fatal human cases may have only a slight effect on the ordinary experimentally susceptible animals (mouse, rabbit), while cocci from mild cases in man may from the outset be extremely pathogenic for these animals. There seems also to be a limit to the degree to which a streptococcus can be exalted by passage through a given species of animal. We have experimented with a large number of streptococci obtained from widely different sources (cases of diphtheria associated with streptococci, ulcerated throats, cases of scarlatina, puerperal fever, abscesses, erysipelas, streptococci obtained from the lungs of pigs dead from hog cholera, &c.). We have found it impossible to detect marked differences in the cultural and morphological characters. Some streptococci have been exalted to a high degree of virulence, others have reached only a medium degree, although a large number of passages have been made. We refer in particular to two varieties. The method adopted was to inoculate rabbits subcutaneously, and cultivations were made after death from the heart blood or the liver. Marmorek's medium as well as ordinary bouillon were used and the cultures incubated for eighteen to twenty-four hours before inoculation into fresh animals. Streptococcus I. was obtained from a severe case of facial erysipelas. The cultures were made directly in ascitic fluid bouillon, and after eighteen hours incubation, were used for inoculations. The animals used were rabbits, 1000-1500 grammes in weight. The results are seen in the following table :

The cultures grew luxuriantly, forming exceedingly long chains. The smallest amount which killed a rabbit was $\frac{1}{10}$ of a cubic centimeter. The effect of the inoculations seemed to be local rather than general. In fact the coccus was not raised to the septicæmic stage. It remained as a pyogenic organism, except in the case of one or two of the rabbits which succumbed to a general infection with only slightly developed local lesion. Experiments with this organism were repeated several times, but the results were not different.

Streptococcus II. was obtained from an acute abscess. Cultures were made in ascitic fluid bouillon and were pure. The results obtained by passage through rabbits contrasts

markedly with the results obtained in the case of streptococcus I.

STREPTOCOCCUS I.

Rabbit.	Dose.	Method of Inoculation.	Result.
1	1 cc. of original culture ..	Subcutaneous. Ear	Ear erysipelas severe. Recovery.
2	·5 cc. culture from incision in erysipelatous swelling of Rabbit 1	Intravenous	Death in four days. Dissemination of cocci throughout the body.
3	·25 cc. culture from liver of Rabbit 2	Subcutaneous	Death in eight days. Abscess at seat of inoculation. Metastases in liver, spleen, and kidneys.
4	·20 cc. culture from liver of Rabbit 3	,,	Local abscess. Recovery.
5	·5 cc. culture from Rabbit 3	Intravenous	Death in four days. Dissemination of cocci throughout the body.
6	·25 cc. culture from Rabbit 5	Subcutaneous	Abscess at seat of inoculation. Recovery.
7	·5 cc. culture from abscess of Rabbit 6	,,	Abscess. Recovery.
8	·25 cc. culture from abscess of Rabbit 7	Intravenous	Death in eight days.
9	·1 cc. culture from liver of Rabbit 8	Subcutaneous	Large local abscess. Death in eight days.
10	·1 cc. culture from Rabbit 9	Intravenous	Death in five days.
11	·1 cc. culture from Rabbit 10	Subcutaneous	Death in three days.
12	·1 ,, ,, ,, 11	,,	Abscess. Recovery.
13	·2 cc. culture from abscess of Rabbit 12	,,	Death in six days.
14	·1 cc. culture from Rabbit 13	,,	Recovery with abscess.
15	·2 cc. culture from abscess of Rabbit 14	,,	Death in four days.
16	·1 cc. culture from Rabbit 15	,,	Death in five days.
17	,, ,, ,, 16	,,	Death in four days.
18	,, ,, ,, 17	,,	Death in four days.
19	·05 cc. culture from Rabbit 18	,,	Abscess. Recovery.
20	·2 cc. culture from Rabbit 18	,,	Death.
21	·1 ,, ,, ,, 20	,,	Recovery.
22	·1 ,, ,, ,, 20	Intravenous	Recovery.

In the course of twenty-six passages the virulence of this streptococcus had become enormously exalted, the doses rapidly diminishing in amount for each successive passage. Thus: $\frac{cc.}{4}$, $\frac{cc.}{10}$, $\frac{cc.}{40}$, $\frac{cc.}{100}$, $\frac{cc.}{200}$, $\frac{cc.}{400}$, $\frac{cc.}{666}$, $\frac{cc.}{1000}$, $\frac{cc.}{2000}$, $\frac{cc.}{5000}$, $\frac{cc.}{8333}$, $\frac{cc.}{10000}$, $\frac{cc.}{20000}$, $\frac{cc.}{25000}$, $\frac{cc.}{33000}$, $\frac{cc.}{50000}$, $\frac{cc.}{500000}$, $\frac{cc.}{1000000}$, $\frac{cc.}{2000000}$, $\frac{cc.}{5000000}$, $\frac{cc.}{10000000}$, $\frac{cc.}{100000000}$.

STREPTOCOCCUS II.

Rabbit.	Dose.	Method of Inoculation.	Result.
1	·25 cc. original culture 18 hours' old	Subcutaneous; skin of abdomen	Dead 18 hours; slight local lesion; dissemination of cocci.
2	·1 cc. culture from liver of Rabbit 1	,,	Dead 14 hours; slight local lesion.
3	·025 cc. culture ,, Rabbit 2	,,	Dead 12 hours; slight local lesion.
4	·01 cc. culture ,, Rabbit 3	,,	Dead 24 hours.
5	·005 cc. culture ,, Rabbit 4	,,	Dead 18 hours.
6	·001 cc. culture ,, Rabbit 4	,,	Recovery.
7	·0025 cc. culture ,, Rabbit 5	,,	Death 28 hours.
8	·0015 cc. culture ,, Rabbit 7	,,	,, 24 hours.
9	·0010 cc. culture ,, Rabbit 8	,,	,, over night.
10	·0005 cc. culture ,, Rabbit 9	,,	,, 36 hours.
11	·0002 cc. culture ,, Rabbit 10	,,	,, 40 hours.
12	·00012 cc. culture ,, Rabbit 11	,,	,, 24 hours.
13	·00010 cc. culture ,, Rabbit 12	,,	,, 18 hours.
14	·00005 cc. culture ,, Rabbit 13	,,	,, 12 hours.
15	·00004 cc. culture ,, Rabbit 14	,,	,, 18 hours.
16	·00003 cc. culture ,, Rabbit 15	,,	,, 36 hours.
17	·00002 cc. culture ,, Rabbit 15	,,	,, 48 hours.
18	·00002 cc. culture ,, Rabbit 15	,,	,, 36 hours.
19	·00001 cc. culture ,, Rabbit 18	,,	,, 36 hours.
20	·000005 cc. ,, Rabbit 19	,,	,, 36 hours.
21	·000002 cc. ,, Rabbit 20	,,	,, 48 hours.
22	·000001 cc. ,, Rabbit 21	,,	,, 3 days.
23	·0000005 cc. ,, Rabbit 22	,,	Alive.
24	·000001 cc. ,, Rabbit 21	,,	Death 4 days.
25	·000001 cc. ,, Rabbit 24	,,	Well.
26	·000001 cc. ,, Rabbit 24	,,	Dead.

One millionth of a cubic centimeter was the smallest amount which produced a fatal result. The limit is reached by the difficulties of thoroughly mixing the suspended cocci in the diluting medium. As a rule the local lesion produced by the inoculation of the virulent streptococci was slight. Sometimes a gelatinous œdema was observed with a few punctiform hæmorrhages. The internal organs showed little abnormality with the exception of the liver, which frequently presented an appearance resembling acute yellow atrophy. The organ was small, of a pale yellow colour, and the liver cells were extremely granular, and in many cases the nuclei had disappeared. The cocci found in the bodies of the rabbits were almost exclusively diplococci. In one experiment the administration of at least a thousand times the minimal fatal dose caused death in a rabbit in *four* hours. No local lesion could be detected, but streptococci were found in all the organs and in the blood in enormous quantities.

Immunisation Experiments.—In the course of nine months a horse was subjected to inoculations of cultures of streptococcus I. The injections were followed by high temperatures and large local swellings. The swellings, without exception, suppurated, healing up rapidly after evacuation of the pus. The pus contained pure cultures of streptococci. The doses varied from 1 cc. to 200 cc. Throughout the entire course of the treatment the micro-organism showed itself to be pyogenic, the condition of the streptococcus most frequently met with in man. Altogether 663 cc. of the cultures were inoculated into the horse. One month after the last inoculation of streptococcus I. the animal received 50 cc. of streptococcus II. from rabbit 13 ($\frac{cc.}{1000}$ fatal dose). A large swelling and high temperature followed, but no suppuration took place. Three weeks afterwards 25 cc. culture from rabbit 21 ($\frac{cc.}{50000}$ fatal dose) was injected. Here again there was no suppuration. A fortnight later the animal received a third injection of 50 cc. of culture from a rabbit which died from $\frac{cc.}{1000000}$. No suppuration followed.

We have thus the remarkable result that an animal

immunised against a weak streptococcus is also to a very large extent immune against a streptococcus exalted to a highly septicæmic condition by passage through rabbits. It might be objected that the passage through rabbits had diminished the virulence for the horse, as Petruschky has shown that increase of virulence for rabbits is associated with decrease for mice. Against this, however, are the results which we have obtained in several other horses. A healthy horse received for the first inoculation $\frac{1}{20}$ cc. of a culture of Streptococcus II. from rabbit 11. The result was a very large swelling with a temperature which reached a maximum of 106°. Had the two streptococci been different in nature, the horse which received 50 cc. of a culture of similar strength would certainly have succumbed.

From these experiments, therefore, we seem to be justified in asserting (1) that the degree to which a streptococcus can be exalted by passage through a susceptible animal varies; (2) that an animal immunised against a streptococcus from a case of erysipelas is also immune against a streptococcus from an abscess.

ON THE SO-CALLED "PSEUDO-" DIPHTHERIA BACILLUS AND ITS RELATION TO THE KLEBS-LÖFFLER BACILLUS.

BY RICHARD T. HEWLETT, M.D., M.R.C.P., AND EDITH KNIGHT, M.B.

VARIOUS opinions have been held as to the nature of the so-called pseudo-diphtheria bacillus, and there is considerable confusion among writers as to its characters, and also as to its relation to the true diphtheria bacillus. It will therefore, be necessary to review briefly the literature of the subject, and then to define clearly in what sense the term "pseudo-diphtheria bacillus" is used in this paper.

Löffler[1] first applied the name, "pseudo-diphtheritic" bacillus to an organism occurring with the true bacillus diphtheriæ, which he differentiated from the latter by the following characters:—It was somewhat smaller, and its ends were less frequently swollen, and its colonies whiter and more finely notched at the margins. The important distinction, however, was that it was non-pathogenic when tested on animals.

Von Hoffman,[2] a few months later, drew attention to an organism which he termed the "pseudo-diphtheria bacillus," and which he distinguished from the diphtheria bacillus by the following characteristics:—It was shorter and its form was more regular, the cultures on blood serum were a purer white than the yellowish-white or pale yellowish of the true diphtheria bacillus, the minimum culture-temperature was

[1] *Centralb. f. Bak.*, ii., 1887, p. 105.

[2] *Wiener med. Wochenschr.*, 1888, No. 3 und 4. Abstr. in Tagebl. d. 60. Versamml. Deutscher Naturf. und Aerzte, in Wiesbaden, 1887, p. 119.

somewhat lower, so that on gelatin a considerable growth is often observed.[1] An essential distinction was its behaviour on agar; a plentiful growth was obtained on plates, while the diphtheria bacillus under similar conditions showed a much feebler growth. It was non-virulent and was commonly found in scarlet fever, measles, pharyngeal catarrh, &c. In a paper entitled "Zum Wesen des Croups und der Diphtherie," Kolisko and Paltauf[2] also drew attention to the difference between agar cultures of the Klebs-Löffler bacillus and Von Hoffmann's bacillus, and stated that the latter was found but seldom. Zarniko,[3] in 1889, examined a number of cases for the diphtheria bacillus—eleven anginas (seven ordinary, three scarlet fever, and one measles), and eighteen with healthy throats but suffering from other diseases (diabetes, rheumatism, &c.). In not one of these was the diphtheria bacillus found, and once only a bacillus identified as the pseudo-diphtheria bacillus of Löffler. This was found to differ from the true diphtheria bacillus by producing no acid when cultivated in broth, while the rods were shorter and plumper, and involution and degeneration forms were but seldom met with. Escherich[4] described similar distinctions between the diphtheria and pseudo-diphtheria bacillus, the colonies of the latter being whiter and bouillon cultures remaining alkaline. It was only found twice out of a number of examinations. Klein,[5] in an elaborate report, stated that he had isolated from diphtheritic membrane two organisms differing from each other in some respects. One of these did not grow on gelatin below 20° and did not grow on potato, while at higher temperatures on gelatin and on agar its colonies were identical with those of the diphtheria bacillus. This he regarded as probably the pseudo-diphtheria bacillus of Löffler.

Roux and Yersin[6] have discussed the question of the

Löffler had stated that the diphtheria bacillus would not grow on gelatin.

[2] *Wiener klin. Wochensch.*, 1889, No. 8.

[3] *Centralb. f. Bak.*, vi., 1889, p. 224.

[4] *Centralb. f. Bak.*, vii., 1890, p. 8.

[5] Supplement to the Annual Rep. of the Local Gov. Board for 1889 (Medical Officer's Rep.) p. 143.

[6] *Ann. de l'Inst. Pasteur*, iv., 1890, p. 409.

pseudo-diphtheria bacillus. They give the following description of the characters of this organism:—Its colonies on blood serum are identical with those of the true diphtheria bacillus, microscopically it is identical, but stains more uniformly with methylene-blue, or shows granules. In alkaline bouillon it forms a deposit on the walls of the vessel, and in this medium frequently shows pear or club-shaped involution forms. It differs from the true diphtheria bacillus in being somewhat shorter; its cultures in bouillon are more abundant, and the change of reaction from acid to alkaline takes place much more rapidly; it will grow in a vacuum, but less readily than the true diphtheria bacillus, and is non-virulent. The pseudo-diphtheria bacillus occurs not unfrequently in simple anginas, and was met with in twenty-six out of fifty-nine children at a school where for some time previously there had been *no* case of diphtheria.

Roux and Yersin found that virulent Klebs-Löffler bacilli could be deprived of their virulence by cultivating in broth for a month at a temperature of 39·5° C. They were unable to restore the virulence to bacilli which had completely lost it, but slightly virulent bacilli regained their full virulence if injected into animals together with the erysipelas streptococcus.

Abbott[1] examined fifty-three patients suffering from various non-diphtheritic throat affections. Of these, forty-nine presented nothing of particular interest. In the remaining four, organisms were isolated which closely resembled the Klebs-Löffler bacillus, and were therefore subjected to detailed study. In three of these cases bacilli were isolated, which, with the exception of being non-virulent, were culturally and morphologically indistinguishable from the Klebs-Löffler bacillus, while in the fourth case the organism resembled the Klebs-Löffler bacillus in every particular, except that it formed a brownish growth on potato and was non-virulent. The author regards the first three forms as non-virulent Klebs-Löffler bacilli, but does not express an opinion as to the nature of the fourth case. Fränkel[2] speaks

[1] *Johns Hopkins Hosp. Bull.*, No. 17, Oct.—Nov., 1891.

[2] *Berlin. Klin. Woch.*, No. 11, 1893.

of the pseudo-diphtheria bacillus as morphologically identical with the virulent forms, and includes the xerosis bacillus under this designation. He considers that the non-virulent pseudo and virulent Klebs-Löffler belong to the same species.

Koplick[1] isolated from four cases organisms which he regarded as "pseudo-diphtheria" bacilli. It is interesting and important to note that these bacilli apparently replaced typical and virulent Klebs-Löffler bacilli on the same locality on the tonsil. The author describes the pseudo-bacillus as growing better on agar, and forming a larger deposit in bouillon, than the virulent diphtheria bacillus, and producing an alkaline and not an acid reaction. Animals were unaffected by 8 cc. of a forty-eight hours broth culture, and were not protected against the virulent diphtheria bacillus by such injections. He does not formulate a definite opinion as to the relation of his pseudo- to the true diphtheria bacillus.

In an elaborate paper, Park and Beebe[2] give a good summary of the literature on the pseudo-diphtheria bacillus and its relation to Klebs-Löffler. They divide the diphtheria-like bacilli into three groups: "(1) Virulent Löffler's bacilli, characteristic in growth, producing acid in bouillon; (2) bacilli identical with Löffler's bacillus in growth, producing acid in bouillon, and having no virulence; and (3) bacilli not having all the characteristics of the Löffler bacillus in growth, producing alkali in bouillon, and having no virulence."

Virulent Klebs-Löffler bacilli were occasionally met with in individuals in perfect health, and with normal throats. Out of 330 healthy throats examined, this was the case in eight; in twenty-four bacilli existed which were in every way identical with the Klebs-Löffler bacillus, except that they were non-virulent to animals. In twenty-seven there were bacilli present, which were so uniform in their peculiarities as to staining, size, and shape, and the production of an alkali instead of an acid, that they could be distinctly

[1] *Med. Jour.* (New York), lix., 1894, p. 300.
[2] *Med. Record* (New York), xlvi., 1894, p. 385.

separated from the Klebs-Löffler bacillus. These bacilli were non-virulent, and did not seem to have any connection with diphtheria. Park and Beebe consider that to these bacilli alone the name "pseudo-diphtheria bacilli" should be given. The plate which accompanies this paper shows very clearly the pseudo-diphtheria bacillus of these authors; it is the same plump, deeply and regularly staining rod which we consider to be the pseudo-diphtheria bacillus. In testing the virulence of this organism the authors used forty-eight hour alkaline glucose broth cultures, an amount of which, equivalent to about $\frac{1}{4}$ to $\frac{1}{2}$ per cent. of the body weight, usually killed within seventy-two hours.[1]

Spronck[2] has experimented with an organism which he describes as the pseudo-diphtheria bacillus, and which he believes to be identical with forms met with by L. Martin.[3] It occurred in four out of 200 cases of anginas, and appeared as a short, thickish rod, which formed colonies whiter, more humid, and with more tendency to spread than the Klebs-Löffler. It developed more abundantly on gelatin, and never showed any distinct acid reaction. In two cases, 1 to 3 cc. of a recent culture, injected subcutaneously into a 300-gramme guinea-pig, produced a little local œdema and considerable general disturbance. In order to test whether these bacilli were really modified diphtheria bacilli or no, Spronck injected animals with diphtheria antitoxin, and subsequently with his pseudo-diphtheria bacillus. Contrary to his expectation, the antitoxin did not modify or annul the disturbance caused by the pseudo-diphtheria bacilli, but, if anything, increased it, and he therefore concludes that this organism is not a modified Klebs-Löffler bacillus. The description given by Spronck of his bacillus is so brief, however, that it is impossible to identify the organism with certainty.

As these pages were going to press, papers by Peters and by Cobbett and Phillips have appeared. Peters[4] gives a care-

[1] The substance of this paper was incorporated in "Report on Bacteriological Investigations and Diagnosis of Diphtheria," Biggs. (*Scientific Bulletin*, No. 1, Health Department, City of New York, 1895, p. 30.)

[2] *La Semaine Médicale*, No. 40, 1896, p. 317.

[3] *Ann. de l'Inst. Pasteur*, vi., 1892, p. 335.

[4] *Journ. Path. and Bact.*, iv., No. 2, 1896, p. 181.

ful description of the varieties of the Klebs-Löffler bacillus, and compares them with the pseudo-diphtheria bacillus of von Hoffmann. With his descriptions we in the main agree, but we regard the Klebs-Löffler bacillus as somewhat more variable than he seems inclined to admit.

Peters, however, does not seem to have noted any difference of growth of the Klebs-Löffler and von Hoffmann bacilli on alkaline potato; perhaps the potato used by him was not so alkaline as was ours. Nor does he lay stress on any *great* difference of reaction exhibited by the two organisms; using neutral litmus sugar agar, we have failed to obtain a trace of acid reaction with the pseudo. He observes that the short form of Klebs-Löffler bacillus, after continued cultivation, may become changed, so as to bear some resemblance to, though it can still be distinguished from, the von Hoffmann bacillus, and concludes that there is no proof forthcoming that the latter is an attenuated form of the diphtheria bacillus.

Cobbett and Phillips[1] were able to compare and identify their cultures of the pseudo-diphtheria bacillus with a culture of this organism obtained from Professor Löffler. They state that the various observers have failed to observe any constant difference, except that of virulence, between the pseudo- and Klebs-Löffler bacilli, and suggest a method of cultivation in litmus sugar broth as a means of differentiation, the former producing an alkaline, the latter an acid, reaction in this medium. They seem, therefore, to have overlooked the papers of Zarniko and Escherich[2] who lay stress on this particular point, which is also mentioned by Günther in his text book, and which, in a modified form, we have found of considerable service in the course of our work. Another point is the extraordinary virulence of the organisms obtained. In one case 0·035 cc. of a forty-eight hours broth culture killed a 350-gramme guinea-pig in forty-four hours; in another, 0·032 cc. killed a 620-gramme guinea-pig in sixty hours. It is curious also that the dose of the least virulent bacillus necessary to kill was only 0·076 cc. We

[1] *Journ. of Path. and Bact.*, iv., No. 2, 1896, p. 193.
[2] *Loc. cit.* (See *ante.*)

have only twice met with a bacillus 0·1 cc. of a forty-eight hours broth culture which would kill in two or three days; most other organisms required 0·25 cc. or 0·5 cc. We believe that this is the experience of other observers, and it would be interesting to know on what factor this extraordinary virulence is dependent. Cobbett and Phillips distinguish the diphtheria bacillus as being virulent and producing acid, the pseudo-diphtheria bacillus as non-virulent and divisible into two classes, acid and alkali producing; the former they consider to be the organism previously described by Roux and Yersin and by Biggs as the pseudo-diphtheria bacillus, and in all probability a non-virulent form of the Klebs-Löffler bacillus, while the latter corresponds with Löffler's pseudo-diphtheria bacillus, which they are inclined to regard provisionally as a distinct species. Again Cobbett and Phillips appear to have overlooked the literature of the subject, for the above classification is practically identical with that suggested by Park and Beebe. (See *ante*.)

This brief survey of the literature of the subject will serve to show that at least two forms have been described under the term "pseudo-diphtheria bacillus." Firstly, there is the organism which morphologically, and to a large extent culturally, is indistinguishable from the Klebs-Löffler bacillus, but is non-virulent, described by Roux and Yersin, Fränkel and Abbot; secondly, there is the organism which, though having a superficial resemblance to, differs materially from the Klebs-Löffler bacillus morphologically and culturally, and is non-virulent, described by Löffler, von Hoffmann, Zarniko, Escherich, Park and Beebe, and Peters. The former we consider is simply a non-virulent diphtheria bacillus, and should not be described as the pseudo-diphtheria bacillus. By the rule of priority this name should be reserved for the organism originally described by Löffler under this title. The organism which we regard as the pseudo-diphtheria bacillus is probably the same as the form described by Löffler under this designation, and almost certainly the same as described by von Hoffmann, Zarniko,

and Peters.[1] It is a shortish rod with rounded ends, its average length being from 1 μ to 1·6 μ, and tending to be slightly thicker at its centre than at the ends. It is non-motile, does not form spores, is arranged in a parallel grouping like the Klebs-Löffler bacillus, and involution forms are, as a rule, not met with. It stains well by Gram's method, and deeply and regularly with Löffler's methylene-blue, segmentation and polar staining being usually absent (see plate I.). On serum, agar, and gelatin it forms cream-coloured colonies or growths, indistinguishable from the Klebs-Löffler bacillus. On ordinary potato it hardly grows at all, what growth there is being quite invisible. On alkaline potato,[2] however, it forms distinct cream-coloured colonies, usually visible by the second day. In stab cultures in gelatin and sugar agar no gas is formed, and the growth is confined to the upper part of the stab. In broth it forms sometimes a granular deposit, sometimes a general turbidity. There is no growth anærobically in hydrogen. On neutral litmus sugar agar a blue colour is developed, indicating the production of alkalinity. Cultivated in peptone water, the indol reaction with sulphuric acid alone can be obtained after a variable time, three to four weeks, while the diphtheria bacillus gives it in about a week; with a nitrite and sulphuric acid, the indol reaction can be obtained both with the pseudo- and diphtheria bacilli in about a week. A broth culture reduces a weak solution of methylene-blue. It does not curdle milk or liquefy gelatin, can be cultivated at from 22° C. to 37° C., and is non-pathogenic to guinea-pigs in doses of 5 cc. of a forty-eight hours broth culture. The differences between this pseudo-bacillus and the Klebs-Löffler bacillus are shown in the following table :—

[1] After reading the papers of Peters and of Cobbett and Phillips, we have no doubt that the organisms described by Löffler, von Hoffmann, and Peters as the pseudo-diphtheria bacillus are identical with the organism which we have regarded as such.

[2] Ordinary potato rendered alkaline with a 10 per cent. solution of sodium carbonate before sterilisation.

TABLE I.

Differences between the Klebs-Löffler and Pseudo-Bacilli.

	Pseudo-Diphtheria Bacillus.	Klebs-Löffler Bacillus.
Morphology	Rods 1μ to 1·6μ in length, tending to be slightly thicker at the centre than at the ends. Is "plumper," shorter and less variable than the Klebs-Löffler Bacillus Involution forms rare	Rods averaging 1·2μ to 2μ in length. Slender, and of same diameter throughout. Considerable variation in size Involution forms usually present
Staining	Stains more deeply and regularly than the Klebs-Löffler Bacillus. Polar staining rare	Staining generally more or less irregular, and polar staining common
Alkaline Potato	Distinct cream-coloured colonies or growth visible in two days	Grows well, but growth is almost invisible
Neutral litmus agar ..	Alkaline reaction	Acid reaction
Stab cultures in sugar agar and gelatin	Growth only at upper part of stab	Growth along whole length of stab
Anærobic cultures in hydrogen	No growth	Grows well
Indol reaction. (Peptone-water cultures, with sulphuric acid alone)	Only after three weeks' growth	After one week's growth

Palmirski and Orlowski[1] stated that the diphtheria bacillus gave the indol reaction after three weeks' growth. As somewhat conflicting opinions have appeared since then, this point was carefully examined with the following result :—

With Sulphuric Acid alone.

	Broth cultures.	Peptone-water cultures.
Klebs-Löffler bacillus	Not up to 19 days' growth	Slight after 7 days' growth
Pseudo-bacillus ..	Not up to 14 days' growth	Only after 21 days' growth

[1] *Centralb. f. Bakt.*, xvii., No. 11, 1895, p. 358.

With Sulphuric Acid and a Nitrite.

	Broth cultures.	Peptone-water cultures.
Klebs-Löffler bacillus	Slight after 6 days' growth	Slight after 4 days' growth
Pseudo-bacillus ..	Slight after 6 days' growth	Slight after 4 days' growth

Peters states that the diphtheria bacillus does not grow anaerobically in hydrogen, whereas we have always obtained good growths. It is true that in the method employed, which was devised by one of us, no special precautions were taken to remove the last traces of oxygen, but by it good growths of tetanus could always be obtained, which seemed a sufficiently satisfactory control.

We will now pass on to consider the conditions under which the pseudo-diphtheria bacillus is met with. The pseudo-diphtheria bacillus occurs not unfrequently in healthy throats and also in anginal conditions in certain diseases, notably scarlet fever. This has been urged as an objection to the view that this organism is a modified Klebs-Löffler bacillus, but as non-virulent and occasionally virulent Klebs-Löffler bacilli also occur in healthy throats—the former sometimes in nearly 50 per cent. of those examined (Roux and Yersin)—the presence of the pseudo-diphtheria bacillus in healthy or anginal throats can hardly be adduced as a proof one way or the other. Our own experience in this direction is limited, and perhaps not altogether free from sources of fallacy. We have examined 84 children in a large school, where some cases of diphtheria had occurred, and on this account the condition may not be considered to be quite normal. Among the 84 children Klebs-Löffler bacilli were found in 2, and the pseudo-diphtheria bacillus in 8, which is nearly the proportion met with by Parke and Beebe (27 in 330). None of these eight children were under treatment before or after examination, and they were distributed among different dormitories. Two of the cases had enlarged glands in addition, and in two others there was

a *suspicion* of deficient movement of the soft palate for a short time. (For these notes we are much indebted to Dr. Gripper, of Wallington.) Next we have a record of a small outbreak of diphtheria in a ward of a general hospital, notes of which were kindly supplied by Dr. Lambert Lack. Three cases of sore throat, which were diagnosed as diphtheria, occurred among the patients, and Dr. Lack then examined the throats of the remaining patients and of the nurses in the same ward. There were in all 32 persons, and of these 7 were found to have Klebs-Löffler bacilli (morphologically) and 2 pseudo-diphtheria bacilli. The virulence of 3 of the 7 Klebs-Löffler cases was tested; two were virulent, 1 cc. killing in two days, the third did not kill in a dose of 0·75 cc.

Lastly, we have examined the records of the cases sent to the British Institute of Preventive Medicine for diagnosis. Practically all these cases were suffering from throats which were suspected to be diphtheritic, or about which there was a doubt. Of 844 such examinations, the pseudo-diphtheria bacillus was met with in 32 in the primary cultures, and in only 3 a mixture of Klebs-Löffler and pseudo-bacilli. In about 340, or 40 per cent. of the cases, the Klebs-Löffler bacillus was found; 145 were subsequently re-examined, once or oftener, and among these the pseudo occurred in 24, or a far larger proportion than previously (1:6 as against 1:24 nearly). We do not care to lay too much stress on this point, as other factors, which we have been unable to take into account, may have been present; but at the same time we cannot help thinking that it is somewhat significant that during convalescence from diphtheria, the pseudo should have occurred four times as often as previously—the inference being that the pseudo replaces the Klebs-Löffler.

Another point of interest in this connection is the manner in which the pseudo replaces the Klebs-Löffler in these cases. Almost always the pseudo appears towards the end, the next examination or so revealing an absence of either diphtheria or pseudo-diphtheria bacilli. In order to show this we give all the twenty-four cases mentioned

above in the following table, which shows the characters of the bacilli found in the various examinations. These cases are entirely unselected, are taken from the records of the British Institute, made at the time of examination, and when there was no thought of their publication, so they may fairly be said to be quite unbiased.

As regards scarlet fever, some writers have found the pseudo-bacillus to be of very frequent occurrence in this disease, which is noteworthy, as diphtheria is probably more often a complication or concomitant of scarlet fever than is usually believed. Thus von Ranke[1] has recently found that of the cases of scarlet fever admitted into hospital during two years, about half were associated with the diphtheria bacillus.

We next give brief histories of some of the cases in which the pseudo-bacillus was found at the primary examination, for notes of which we are indebted to Surgeon Bate, R.N., Drs. G. B. Batten, T. H. Cheatle, Fleet-Surgeon Conway, R.N., Drs. J. Roberson Day, Louis F. Dods, F. C. Fisher, Hingston Fox, T. P. Gostling, E. L. Jacob, Henry Malet, H. B. Osburn, J. H. Philpot, and Sandford Smith.

Case 1.—Female, aged 13. Scarlet fever some years ago, sore throat two days, temperature 101°-102°. Small patch of membrane on right tonsil, removal of which was followed by bleeding, swallowing painful, no albuminuria. Throat got well in three days, no complications, no prostration, no sequelæ. No history of infection.

Case 2.—Child, aged —. January 20, sore throat with slight membranous exudation on right tonsil, enlarged gland at the angle of the jaw. January 24, slight membranous exudation on left tonsil, the right clear. On the 26th, practically well. No constitutional disturbance, no albuminuria, no sequelæ. A child had died from genuine diphtheria in the same row shortly before.

Case 3.—Female, aged 77. January 19, sore throat; 20th, patches on tonsils; 21st, patches spreading to soft palate. Antitoxin was injected, very little reaction, the patches persisted until February 7. Recovered. No complications.

[1] *Münch. med. Woch.*, October 20, 1896.

TABLE II.

Case.	First Examination.	Second Examination.	Third Examination.	Fourth Examination.	Fifth Examination.
1. B.A. ..	D	D	P	P	N
2. M.B. ..	D	P + D	D	—	—
3. E.B. ..	D	P + D	N	—	—
4. B. ..	D	P	N	—	—
5. J.C. ..	D	P	P	N	—
6. A.C. ..	D	P	—	—	—
7. F.C. ..	D	P	D	—	—
8. S.C. ..	D	D	P	N	—
9. J.F. ..	D	D	P	N	—
10. J.G. ..	D	P + D	N	—	—
11. E.G. ..	D	D	P	N	—
12. H.G. ..	?	P	D	P	—
13. C.H. ..	D	P	—	—	—
14. D.L. ..	D	P	P	N	—
15. M. ..	N	P	N	—	—
16. M.B. ..	D	P	N	—	—
17. L.P. ..	D	P	N	—	—
18. O.P. ..	D	D	P	N	—
19. M.P. ..	? D	P	N	—	—
20. J.S. ..	D	P	D	N	—
21. A.S. ..	D	D	P	D	N
22. A.T. ..	D	D	P	N	—
23. W. ..	D	P	—	—	—
24. F.W. ..	D	P	N	—	—

D = diphtheria bacilli; P = pseudo-diphtheria bacilli; D + P = a mixture of these; N = neither found.

Case 4.—December 29, 1895. Dr. D. was called to see a child, G. E. L., junr., aged 9 months. On January 6, 1896, he also saw the child's father, G. E. L. On January 7, both were certified as suffering from scarlet fever and removed to the Isolation Hospital. On January 8, the Medical Officer of Health called at the house, and found Mrs. N. suffering from a slight sore throat. On the same day the Medical Officer of Health suspected that the child, G. E. L., was suffering from diphtheria, and a bacteriological examination proved this to be the case. Hearing of this, the Medical Officer of Health again visited Mrs. N., on January 17, and had a swabbing from her throat examined, and pseudo-diphtheria bacilli were found. Two of her children were found to have sore throats, but were not examined. Between January 17 and February 13, Mrs. N. had an attack of inflammation of the kidneys with dropsy; with this exception, Mrs. N. has been well. No sequelæ.

Case 5.—Private ———. February 20, with characteristic wash-leather patches on his tonsils, debilitated, somnolent, with a temperature of 102°, and maxillary glands swollen. The throat cleared under treatment (no antitoxin). March 5, returned to duty. April 11, no sequelæ.

Case 6.—Child, aged 5. May 18, commenced with considerable constitutional disturbance, temperature 103°. Small discrete white patches the size of hemp seed appeared on both tonsils during the following forty-eight hours. Not seriously ill, a considerable trace of albuminuria on the third day. The glands were moderately swollen. No sequelæ.

Case 7.—Boy. Clinically follicular tonsilitis, but more constitutional depression than would have been expected. His strength did not return, and subsequently muscular weakness with decrease of knee-jerks appeared. The palate moved sluggishly, and a sensation of pins and needles all over the body, amounting almost to pain, was experienced. No antitoxin.

Case 8.—Child. The only symptom was a slight croupy cough. No temperature and no visible membrane. Four days previously the child had visited at a house where there were children with "croup."

Case 9.—A. W., aged 9. A schoolboy living in a crowded neighbourhood. Suffered with headache and sore throat for four days (April 27), restless nights and vomiting at commencement. Temperature 99°. The tonsils were swollen and red, with small, pale, filmy spots upon them. The glands in his neck swollen. July 1, reports that he was only laid up a week. No other cases followed and no sequelæ.

Case 10.—L. P., aged 4½ years. April 23, throat with a *suspicion* of membrane on it; it bled readily. Temperature 100·6° (April 24), a patch of membrane on the left tonsil, nasal discharge, the glands in the neck, axillæ, and groin were enlarged. No albuminuria. On April 25, throat clear, nasal discharge much less. May 7, knee-jerks present and no sign of paralysis. May 15, convalescence uninterrupted.

Case 11.—H. W., aged 8 years. March 22, a sudden onset with vomiting. One tonsil very swollen, with two whitish specks, the pharynx red and raw-looking. Temperature 101·2°. March 23, both tonsils were covered with whitish specks. Temperature 100·0°. The condition slightly improved. The case was notified as one of diphtheria, and removed to one of the hospitals of the Asylums Board. On admission there the medical officer in charge reports that there was definite membrane on each tonsil, but no marked constitutional disturbance, no enlarged glands, no albuminuria, no complications, and no sequelæ (treated with antitoxin). Dr. Dods gives the following remarkable history of the residence where this boy lived. In the same rooms (two), T. H. had enteric fever; he recovered, and was removed with diphtheria; he recovered again, and was again removed with diphtheria. A. J. was removed twice and E. J. once with diphtheria. These rooms were then disinfected, white-washed, and re-papered, and then the case of H. W. occurred. The caretaker living below these rooms lost two children in forty-eight hours, with a history of illness, sudden onset bad throats, vomiting, and high fever. A third child was removed with diphtheria. A family living above these rooms had three children removed with diphtheria. The sanitary arrangements were perfect, and other houses of the same block were free.

Case 12.—F. L. M., a strong active boy, aged 3½ years. Had slight chronic pharyngitis. The first week in April he suffered with slight ordinary naso-pharyngeal catarrh, apparently contagious. A week after he complained of sore throat. Temperature 101° to 102°. A swelling in the sub-maxillary region, and both tonsils swollen; on the right one, two small spots of deposit, creamy-white in colour, were observed. He was fretful and somnolent, and on the second day had an attack of collapse; he became very white, and almost pulseless, but quickly rallied. Soon convalesced rapidly, but although strong and active previously, he was very weak for a few days, and hardly able to stand.

Case 13.—Private E. P., November 7, right otorrhœa. Soft

palate became congested. November 13, patches of membrane on both tonsils; glands below the jaws enlarged. Temperature, 103·6°. Antitoxin injected. November 19, throat has slowly cleaned, only some congestion remaining. No albuminuria. No complications. No sequelæ. (Remained under observation until December 18.)

Case 14.—Child. Mild throat attack, with distinct membrane, bleeding on removal of this. Good recovery. No complications. No sequelæ.

These histories seem to show that the pseudo-bacillus is associated with mild anginal conditions, which are free from complications, end in recovery, and are not followed by sequelæ. In many of the cases the anginal condition was associated with distinct patches of membrane. In two of the cases (7 and 12) symptoms were present suggestive of the toxæmia which is met with in diphtheria.

A consideration of the conditions under which the pseudo-diphtheria bacillus occurs, although of considerable value, will hardly determine whether it is a modified Klebs-Löffler bacillus or no; this important point can only be settled by the experimental transformation of the one form into the other. A large number of experiments were therefore performed, with a view to accomplishing this, and we give first of all the details of the apparent alteration of a typical and virulent Klebs-Löffler bacillus into a typical pseudo one.

The bacillus selected for experiment was a very virulent one; 0·25 cc. of a broth culture killed a guinea-pig in twenty-four hours; 0·1 cc. killed a guinea-pig in three and a half days. In microscopical appearance it was almost constantly a very large segmented and polar-stained form. It gave the usual diphtheritic reactions, turning neutral litmus agar red, and giving a good growth anaerobically. Four broth cultures were prepared, and after forty-eight hours' growth were kept for four hours, six and a half hours, seventeen hours, and twenty-four hours respectively at a temperature of about 45° C. The result of heating the culture for twenty-four hours appeared to be that all the bacilli were killed, as no sub-cultures could be obtained.

From the cultures heated four hours and six and a half

hours, sub-cultures were readily obtained. The effect produced by the heating appeared to have been inappreciable, as, inoculated in doses of 0·25 cc. and 0·5 cc., they killed guinea-pigs in thirty to thirty-six hours. The growth in the first sub-cultures obtained from the broth heated for seventeen hours was very poor, showing that most of the organisms had been killed; and the nature of those that remained alive appeared to have been completely changed, as will be seen from the details given below. In later sub-cultures the growth was as good as before heating, and to these the usual tests were applied. The results were as follows:—

Microscopical Characters.—The bacilli were now short; they had lost their polar staining and segmentation, and appeared as short, deeply-stained bacilli, regular in form and staining—in fact, as typical "pseudo-diphtheria bacilli." This appearance has been maintained in sub-cultures up to the present time (the sub-cultures have been carried on for nine generations from the heated broth).

Sub-cultures on Various Media.—To the naked eye the cultures appeared much the same as previously on most of the media. On alkaline potato, a visible growth, consisting of distinct whitish colonies, was obtained.

Reaction to Neutral Litmus Sugar Agar.—This medium was turned distinctly blue; the change was visible in two days.

Anaerobic Cultures.—Broth cultures in hydrogen were incubated for nine days. There was no trace of growth; the broth remained perfectly clear. As a control, a second anaerobic culture was made, and on the same day an anaerobic culture from the original stock (unheated). The difference was very obvious at the end of twenty-four hours, growth being visible in the culture from the original stock, while the other broth was perfectly clear. The difference became more marked later; the flasks were kept for twelve days, at the end of which time there was still no growth in the sub-culture from the heated stock, while in the other it was very good.

Indol Reaction.—Whereas the original unheated stock,

cultivated in peptone water, gave the indol reaction with sulphuric acid alone after six days' growth, the heated one, under the same conditions, did not give the reaction in less than a fortnight.

Inoculation Experiments on Guinea-pigs.—Broth cultures were inoculated into guinea-pigs at various times, in doses varying from 0·25 cc. to 5 cc. In all cases they remained perfectly well. The last guinea-pig has thus received a dose fifty times as great as that previously found to be lethal for the unheated stock; but the virulence of the bacilli appears to be quite lost—or, at all events, enormously diminished.

It would thus seem that a virulent Klebs-Löffler bacillus has been changed into a pseudo-bacillus typical in all its reactions and in pathogenesis. It might be suggested that the original stock was a mixture of Klebs-Löffler and a few pseudo, and that the heating destroyed the Klebs-Löffler, leaving the pseudo. Against this it may be pointed out that the original stock never showed any pseudo-forms in *any* of the sub-cultures, and granting—as one must do in order to accept this explanation—that the pseudo is more resistant than the Klebs-Löffler, we should have expected the pseudo to have made its presence visible sometimes, which it never did. Personally, we feel convinced that this explanation is untenable, and we are satisfied that the virulent Klebs-Löffler was converted into the pseudo. We have attempted to obtain the same transformation with other virulent diphtheria bacilli, but not so successfully, although in two instances, after the heating pseudo-forms were obtained, the change from Klebs-Löffler to pseudo was not nearly so complete. The amount of heating required seems to be a very delicate factor; too little leaves bacilli comparatively unaltered, a little more kills them completely. We have also attempted the reverse transformation, viz., that of the pseudo into the Klebs-Löffler bacillus.

Conversion of the Pseudo-Diphtheria Bacillus into the Klebs-Löffler Bacillus.

It will be generally acknowledged that the conversion of a non-virulent organism into a virulent form is by no means

an easy matter, and this difficulty is enormously increased when the organism is absolutely non-pathogenic. On this account we have experienced the greatest difficulty in transforming the pseudo-diphtheria bacillus into a virulent form. We believe, however, that we have succeeded in accomplishing this in one or two cases, and, having now devised this method, we shall hope before long to be able to report other instances. In the first case, the pseudo-bacillus was obtained from the throat of a nurse who had been nursing a case of diphtheria. The following is the history, for which we are indebted to Dr. Hingston Fox :—

"Miss ——, aged 28, had been nursing a case of undoubted diphtheria (Mrs. ——, clinically, severe diphtheria, followed by paresis; diphtheria bacilli found bacteriologically). Miss —— had gone away for a short rest, and before returning to a house where there were children, being somewhat unwell; *although having no throat symptoms*, a swabbing from the throat was sent for bacteriological examination, and the pseudo-bacillus found. No further ailment followed, and she was soon quite well." (Case 10, Table III., p. 29.)

The culture from the throat showed typical pseudo-bacilli. Sub-cultures showed usually typical pseudo, occasionally a few bacilli resembling Klebs-Löffler bacilli were met with among the pseudo, but otherwise it behaved as a typical pseudo to all the tests. On this account the organism was passed through agar plates, and sub-cultures prepared from single isolated colonies. From the first sub-culture after the agar plates a broth culture was prepared, and, after growing for forty-eight hours, 2 cc. were injected subcutaneously into a 200 gramme guinea-pig. This failed to produce any effect, the animal remaining perfectly well. The first, second, and third generations after the agar plates showed only typical pseudo-forms. In the fourth generation, however, bacilli of a distinctly short Klebs-Löffler type were observed. The fifth generation was again pseudo, the sixth diphtheritic, the seventh a mixture of somewhat pseudo and somewhat diphtheritic, the eighth Klebs-Löffler with polar staining, the ninth short diphtheritic without polar staining. From the tenth generation a broth cul-

ture was prepared, and a guinea-pig inoculated with 2 cc. without result, the animal remaining well. The organism was again passed through agar plates, and the first three generations after these showed Klebs-Löffler forms. From the second sub-culture a broth culture was prepared, and a guinea-pig inoculated with 2 cc. was found dead at the end of ten days. After this, the sub-cultures for eighteen generations were variable, some showing typical Klebs-Löffler forms, others as typical pseudo. From the eighteenth sub-culture broth cultures were prepared, and two guinea-pigs were inoculated with 4 cc. and 5 cc. respectively, but remained perfectly well. The same sub-culture reacted in every way as a typical pseudo: it gave an alkaline reaction, strictly aerobic growth, and visible colonies on alkaline potato.

Up to this point the cultures were usually incubated eighteen to twenty hours, occasionally forty-eight hours, on serum at 37° C. Now, however, a new departure was made, the nineteenth and twentieth generations being serum cultures incubated at 37° C. for seven days each. From the twentieth generation a broth culture was made and incubated for *six days*; 5 cc. were then inoculated into a 250 gramme guinea-pig, which died in thirty-six hours. There was marked œdema at the seat of inoculation, and cultures were obtained. This guinea-pig may be described as No. I.

From this same broth culture which proved fatal to guinea-pig No. I., a second broth culture was prepared, incubated for forty-eight hours, and 4 cc. injected into a 316 gramme guinea-pig (No. II.). This died in about forty hours, with marked œdema at the seat of inoculation, and cultures were obtained. The cultures obtained from guinea-pigs I. and II. showed typical Klebs-Löffler bacilli. From the second generation obtained from guinea-pig No. II., broth cultures were prepared, and two large guinea-pigs—536 (No. III.) and 478 grammes—were inoculated with 4 cc. and 2 cc. respectively. Both died in thirty-six to forty hours, and cultures obtained from the seat of inoculation showed typical Klebs-Löffler bacilli.

Broth cultures were prepared from the first generation

from guinea-pig No. III., and two small guinea-pigs, inoculated with 1·0 cc. and 0·5 cc. respectively, died in about twenty hours, and pure cultures were obtained from both.

Broth cultures were also prepared from the second generation from guinea-pig No. I., and two large guinea-pigs (690 and 470 grammes) inoculated with 3 cc. and 1 cc. respectively, died in thirty to thirty-six hours. Cultures obtained from the seat of inoculation showed typical Klebs-Löffler bacilli. From the fifth generation from the same guinea-pig (No. I.), broth cultures were prepared, and two small guinea-pigs, inoculated with 0·5 cc. and 0·25 cc. respectively, died in twenty-six hours. Up to the incubation of the serum cultures for a week, no trace of acidity could be detected on cultivation on neutral litmus sugar agar, in fact such cultures were always distinctly alkaline. A sub-culture from the incubated broth culture (incubated for six days and prepared from the incubated serum cultures) used for the inoculation of guinea-pigs I. and II. showed a trace of acidity on the litmus medium after a week's growth. Cultures from the guinea-pigs, however, were strongly acid, turning the litmus medium a bright red in twenty-four hours; they also gave a luxuriant growth anaerobically, and an invisible growth on alkaline potato. These results, it is needless to say, were confirmed on more than one occasion.

In other cases, which showed only typical pseudo-forms both morphologically, culturally, and in non-virulence, similar treatment, viz., by incubating on serum for a week for a variable number of generations, has resulted in broth cultures a week old, inoculated in 5 cc. doses, killing the guinea-pigs in seven to ten days, or a little longer. This result is so constant, and has been repeated so many times, that we are convinced that it is not merely accidental, especially as guinea-pigs inoculated with similar doses from unincubated cultures usually remained perfectly well, and there has been no corresponding mortality among the other animals in the same house.

It might well be objected that continued cultivation and incubation, as described above, are not calculated to increase

TABLE III.

Diphtheria and Pseudo-Diphtheria Bacilli.

	Morphology (on blood serum).		Neutral Litmus Sugar Agar.	Alkaline Potato.	Anaerobic Cultivation in H.	Pathogenesis.
	Primary Culture from the Throat.	Subsequent Cultivations.				48 Hours Broth Cultures.
Case I. (B.)	Typical Klebs - Löffler, medium segmentation and polar staining	Typical Klebs-Löffler	Acid	—	Good growth; commencing in 24 hours	1 cc. killed a 655 gram. guinea-pig in 36 hours
Case II. (L.)	Typical Klebs - Löffler, medium, with polar staining	Typical Klebs-Löffler	Acid	Invisible growth	Ditto	0·25 cc. killed in 24 hours; 0·1 cc. killed in 80 hours
Case III. (V.)	Diphtheritic. Segmentation and polar staining almost absent	Diphtheritic	Acid	Generally invisible, sometimes slight	Ditto	2 cc. killed; 1 cc. did not kill
Case IV. (P.)	Large Klebs-Löffler, with marked segmentation and polar staining	Klebs-Löffler	Acid	Invisible growth	Slight growth in 48 hours, more later, but not so abundant as in preceding cases	1 cc. killed 500 gram. guinea - pig in 36 hours, 0·2 in 200 gram. guinea-pig died in ten days
Case V. (H.)	Typical Klebs - Löffler large	Occasionally distinctly pseudo forms	Acid	—	Good growth; commencing in 24 hours	0·5 cc. killed a 365 gram. guinea-pig in 60 hours
Case VI. (J.)	Large Klebs-Löffler	Klebs-Löffler	Acid	—	Ditto	0·5 cc. killed a 500 gram guinea - pig in 36 hours, 0·25 cc. a 445 gram. guinea-pig in 54 hours

Case VII. (T.)	Klebs-Löffler, medium	Klebs-Löffler	Acid	—	Ditto	0·5 cc. killed a 554 gram. guinea-pig in 36 hours
Case VIII. (W.) Clin. diagnosis, Follicular Tonsilitis	Diphtheritic, but not at all typical. In fact diagnois somewhat doubtful	Generally somewhat pseudo, occasionally diphtheritic	Acid	Invisible growth	Slight growth in two days, fair later	2 cc. did not kill
Case IX. (B.) From throat two and a half weeks after tracheotomy	Short form. Some diphtheritic, others "pseudoish"	Generally Klebs-Löffler, occasionally "pseudoish"	Slightly acid	Visible growth	Ditto	1 cc. and 2 cc. did not kill. Guinea-pigs inoculated with 5 cc. died in about 14 days
Case X. (F.)	Typical pseudo	Sometimes pseudo, sometimes Klebs-Löffler	Alkaline	Visible growth	No growth	1 cc., 2 cc. and 5 cc. did not kill
Case XI. (B.) Seven weeks after Diphtheria	Typical pseudo	Occasionally Klebs-Löffler	Alkaline	Visible growth	No growth	4 cc. did not kill
Case XII. Scarlet Fever Throat	Typical pseudo	Occasionally typical Klebs-Löffler	Alkaline	—	No growth	—
Case XIII. (W.) Three months after Diphtheria	Typical pseudo	Occasionally slightly diphtheritic	Alkaline	—	No growth	1 cc. did not kill
Case XIV. (L.)	Typical pseudo	Occasionally slightly diphtheritic	Alkaline	—	No growth	1 cc. and 2 cc. did not kill, 5 cc. *incubated* culture killed in 14 days
Case XV. (M.)	Typical pseudo	Occasionally some diphtheritic forms	—	—	—	5 cc. did not kill
Case XVI. (E.).. ..	Typical pseudo	Ditto	Alkaline	—	No growth	—
Case XVII. (N.) ..	Typical pseudo	Ditto	Alkaline	—	No growth	—

the virulence of an organism, but rather the reverse. We notice, however, that Peters found that the virulence of one of his short diphtherias, after cultivation for a year and a half, was greater than that of the original stock, and we have obtained similar results by our method in the case of two slightly virulent diphtheria bacilli. One, morphologically Klebs-Löffler, did not kill in less than 2 cc., and that only after several days, but after treatment it became sufficiently virulent for 1 cc. to kill in two to three days. The other, although usually diphtheritic, was occasionally pseudoish, morphologically; it gave a slightly acid reaction and fair growth anaerobically, but a visible growth on alkaline potato, and 5 cc. did not kill under fourteen days. By cultivation and incubation for a number of generations, 2 cc. of a forty-eight hours' broth culture kills in about three or four days.

The result of these experiments would seem to show that pseudo-bacilli may be converted into Klebs-Löffler forms. It may again be suggested that the cultures employed were really mixtures of pseudo and non-virulent Klebs-Löffler forms, the latter gradually supplanting the former and becoming virulent, especially as the Klebs-Löffler forms were frequently seen in the sub-cultures. As a matter of fact, however, we have observed the same thing in eight cases of what were originally typical pseudo, which have been studied at some length; in the various sub-cultures, diphtheritic forms have always appeared occasionally. This will be seen in the Table III., in which the characters of several Klebs-Löffler and pseudo-bacilli are tabulated (see previous pp.).

If the occasional occurrence of Klebs-Löffler or diphtheritic forms in cultures of what originally appeared to be typical pseudo be held to show that these cultures were mixed from the first—and as we have hardly ever failed to find this—we must admit that Klebs-Löffler forms are generally associated, in a larger or smaller proportion, with the pseudo, and that cases in which the latter occur are therefore to be regarded with suspicion, being in the majority of cases associated with a small number of diphtheria bacilli.

Another point brought out by this table is that the Klebs-Löffler form is apparently far more stable than the pseudo—pseudo forms rarely developing from the former.

A third point is that there are intermediate forms—connecting links, as it were, between the Klebs-Löffler and the pseudo-bacilli.

Thus, first of all, there are the typical and virulent Klebs-Löffler bacilli (Table III., Cases 1, 2, 4, 5, 6, and 7); next there are bacilli not so typical, but still diphtheritic and of medium virulence (Table III., Case 3); then diphtheritic bacilli, which frequently show pseudo-forms, and are of slight virulence (Table III., Cases 8 and 9); then pseudo-bacilli, non-virulent, but with which Klebs-Löffler or diphtheritic forms are frequently intermixed (Table III., Cases 10, 11, and 12), and, lastly, the most typical pseudo-bacilli (Table III., Cases 13, 14, 15, 16, and 17). A consideration of the various points raised in this paper leads us to the conclusion that the so-called pseudo-diphtheria bacillus is frequently a modified and non-virulent diphtheria bacillus. We cannot say that all pseudo-bacilli are modified diphtheria bacilli, for possibly there may be more than one form of pseudo; but there is no doubt that the diphtheria bacillus may assume the form of the pseudo-bacillus, and we therefore think that cases in which the pseudo is isolated should be regarded as diphtheritic, and as possible sources of infection, though the prediction may be pretty safely made that such will be mild and end in recovery.

As regards the position in the scale of virulence of the pseudo, just as we can have a very virulent organism, so we can have a very non-virulent one, and we consider that the pseudo belongs to this category, and that it is a diphtheria bacillus, far removed from virulence, while the non-virulent Klebs-Löffler forms (the pseudo of Roux and Yersin) we should consider to be slightly on the negative side of the border line between non-virulence and virulence.

In conclusion, we give the following summary of the points dealt with in this paper :—

(1) At least two forms have been described under the term pseudo-diphtheria bacillus, (*a*) one, in morphology a

Klebs-Löffler bacillus, but non-virulent (Roux and Yersin, &c.), and (*b*) another, shorter, plumper, and more regular in form and staining more uniformly than the Klebs-Löffler bacillus (Löffler, von Hoffmann, Park, Beebe, Peters, &c.).

(2) The term pseudo-diphtheria bacillus, should be reserved for the latter form.

(3) This pseudo-bacillus differs from the Klebs-Löffler bacillus in morphology and staining, by producing an alkaline and not an acid reaction, by giving a visible growth on alkaline potato, by not growing anaerobically, and by being non-virulent.

(4) The pseudo-bacillus may occur in healthy throats, and in various diseases is associated with mild anginal conditions which usually run a favourable course, and are not followed by sequelæ.

(5) The pseudo-bacillus seems to replace the diphtheria bacillus in some cases of diphtheria as they progress towards convalescence.

(6) Pseudo-forms may occur, but very rarely, in cultures of the Klebs-Löffler bacillus.

(7) Klebs-Löffler, or diphtheritic forms, occur almost always some time or other in cultures of the pseudo-bacillus.

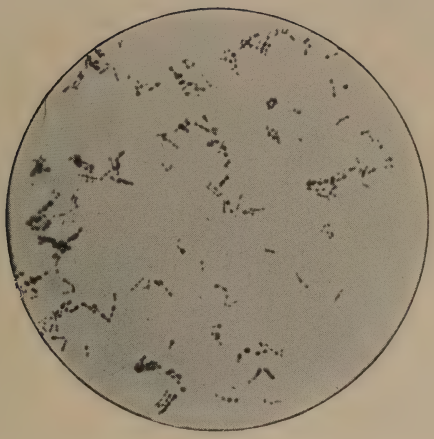
(8) In a number of cultures it is possible to obtain a series of organisms which form a connecting chain between the Klebs-Löffler and the pseudo.

(9) By careful heating it has apparently been possible to convert a typical virulent Klebs-Löffler into a typical non-virulent pseudo-bacillus.

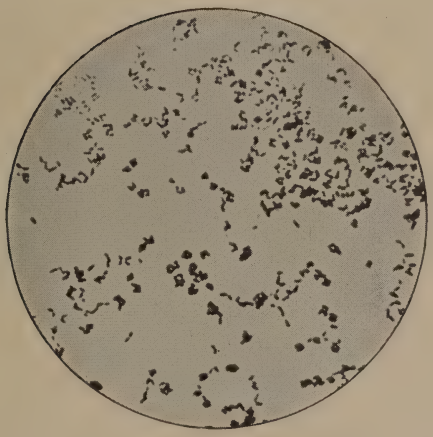
(10) By cultivation and incubation and passage through an animal, a pseudo has apparently been converted into a Klebs-Löffler bacillus.

(11) We therfore consider that the pseudo is sometimes a modified Klebs-Löffler, though perhaps not always, as possibly more than one species having the same morphology may exist.

We have to express our thanks to all those gentlemen who have kindly furnished us with notes, and to Dr. Macfadyen for allowing us to make use of the records of the British Institute of Preventive Medicine, and for the interest he has taken in our work.



Klebs-Löffler bacillus. Twenty hours' serum culture.
Löffler's blue x 1000.



Pseudo-diphtheria bacillus. Twenty hours' serum culture.
Löffler's blue x 1000.

NOTE ON A PECULIAR MOVEMENT OF CERTAIN INTRA-CELLULAR PARTICLES IN YEAST CELLS.

BY WM. ST. C. SYMMERS, M.B.Aberd.

In the autumn of 1893, while engaged in an attempt to cultivate the microsporon furfur from cases of pityriasis versicolor, I obtained from among the branny scales of this disease an organism which belonged to the class of the budding fungi. This yeast-like fungus grew readily in all the usual culture media of the laboratory, and although as a rule it reproduced itself by gemmation, it also, under certain conditions, produced long jointed thread-forms resembling very closely the similar mycelial growth of the thrush-fungus (*Soorpilz*). When grown in sugar-free bouillon, this organism spread over the surface of the fluid in twenty-four hours, and had a peculiar white, perfectly dry appearance like so much white powder lying on the surface of a clear limpid liquid. On agitation this surface growth showed itself fragile, easily crumbled and sank through the fluid, remaining at the bottom of the flask as a whitish deposit which could easily confer, on shaking the flask, a misty turbidity to the supernatant fluid. However, in a few minutes the turbidity of the bouillon passed away, the cloud settled into sediment, and the surface of the fluid in another twenty-four hours again became covered by the dry powdery layer. This peculiar growth in bouillon was the most marked naked-eye appearance possessed by this fungus. On examining microscopically the dry surface growth it was found to consist entirely of spherical or oval cells, of 8-12 μ in diameter, doubly contoured, containing vacuoles, and very frequently a highly refractile globular body. Many of these

cells were actively budding. The cells stained deeply with the usual solutions of basic anilin dyes, the dye being instantaneously taken up by the cells, which then appeared as deeply stained homogeneous masses. But when a weak solution of the dye was used—for example, when the ordinary laboratory solutions of gentian violet were diluted four or five times with water so that a faint but distinct violet was obtained—the cells took up the dye much less intensely, and the details of their structure were beautifully seen. The usual appearances of yeast cells under these conditions are universally known, and therefore I shall at once proceed to draw attention to the phenomenon which is the subject of this note.

When a loopful of such a surface culture from bouillon is mixed with a drop of water and covered by the cover-slip, and a drop of a weak solution of gentian violet added, it will be found that one or two cells among the number seen in the field of the microscope contain a multitude of minute point-like bodies which are deeply stained by the dye, and which are in a state of extremely active movement. Occasionally it requires some patience and searching over many fields before one such cell is found, although many cells are passed by in which there are similar deeply stained points which, however, are quiescent. When a cell containing these moving particles is watched continuously, it will be seen that the particles apparently touch each other repeatedly, rebounding after such contact, receding to the parietes of the cell, again advancing with a quivering movement, or dancing irregularly throughout the whole interior of the cell, and at times even sweeping around parallel to the wall of the cell. The most generally seen movement is an inextricable intertwining or interdarting of the individual particles. In a few moments it is seen that the particles stick together in twos or sometimes more, still continuing their rapid movement, with, however, some retardation, so that the movements are more easily followed. There thus results a smaller number of particles, each being now increased in size, and moving with a somewhat slower action. Again there is seen an amalgamation of these larger par-

ticles, and this process continues until three or four large particles alone remain to represent the great number of minute points with which the phenomenon began. Ultimately these larger particles also run together into one deeply stained irregular mass, which has a sluggish, oftentimes rolling motion. This mass now almost fills the

FIG. 1.—S. litogenes. Stained with weak gentian violet; showing the same cell with moving particles, which gradually run together and become quiescent in one large particle.

interior of the cell, and occasionally shows a slow swinging, or nodding movement, or nutation of one of its irregular prominences. Or the mass may be quite regular in contour and slowly tumbles in the interior of the otherwise perfectly still cell. As this slow movement gradually ceases the dye, hitherto restricted to the moving particles, now somewhat rapidly spreads over the whole of cell and precludes any further observation of the cell contents. The whole yeast-cell is now deeply stained,

FIG. 2.—Drop culture 14 days. Organism cultivated from the skin in pityriasis versicolor.

and appears as a uniform violet mass, oval or circular in form, and surrounded often by an unstained, thickish investing membrane. These moving particles were several times seen in cells to which was attached a bud in which similar but quiescent granules were present. It occa-

sionally happens that the larger particles resulting from the fusion of the smaller ones become quiescent without forming a single mass, and thus we see the cessation of the movement and accompanying diffusion of the dye take place while two or four or more large particles are still ununited. When examined from day to day in a hanging drop this fungus grows out into thread-forms, forming a tubular, segmented mycelium from which very numerous yeast-like cells bud out in clusters (see figs. 2 and 3). In the various segments similar moving particles are found from time to time, but here the movement is less active and the particles appear large and most frequently devoid of

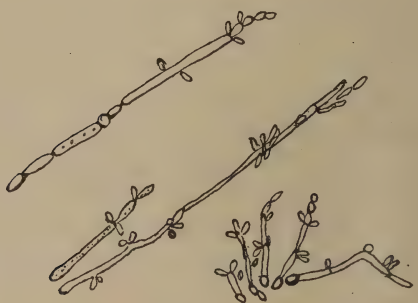


FIG. 3.—From drop culture in bouillon, periphery of the drop 5 days old, 7 ob., 4 oc. Organism cultivated from skin in a case of pityriasis versicolor.

motility. Unfortunately during my absence from the laboratory the cultures of this fungus were destroyed. It is, however, in all probability an oidium and thus a transition form between the hyphomycetes and blastomycetes. However, since 1893 I have very frequently seen a phenomenon in all respects similar to the one indicated above. A systematic examination of various species of saccharomyces has revealed in all cases the presence of these chromatic particles within certain of the cells, and in every yeast so far examined I have been able to demonstrate with more or less facility the presence of actively moving, easily stained particles which gradually run together and ultimately come to rest either in one or more large, deeply stained

masses, the whole process occurring in an otherwise non-motile cell. The yeasts in which I have seen this phenomenon are the following :—

Saccharomyces Anomalus.
,, Ellipsoideus.
,, Membranifaciens.
,, Pastorianus I.
,, ,, II.
,, Exiguus.
,, Litogenes (Sanfelice).

Also in a red yeast which occurred as a contamination on an old culture plate. I am indebted to Dr. W. C. C. Pakes, of Guy's Hospital, for various of the yeasts examined, and in addition to some of those mentioned above, Dr. Pakes gave me three other cultures whose exact identity is not determined, and in these the moving particles were easily found.

The S. litogenes of Sanfelice[1] was one of the best for studying the phenomenon. This organism was found by the Italian observer in the swollen lymph glands of a bullock which died of primary cancer of the liver, and received its distinctive name from its property of forming calcareous masses in the bodies of such animals as guinea-pigs, sheep, dogs, hens, &c. It grows luxuriously in the usual culture media, and on agar-glucose, for example, forms an abundant growth in twenty-four hours, from which cells can be obtained which show the moving particles to perfection. In one of these cells there was a single small particle which was kept under observation, in an unstained state, for eight hours, during which time it moved with great activity to all parts of the cell; what its ultimate fate was I am unable to say. In this particular cell, and indeed in many of the cells showing the phenomena, there is an absence of the large, highly refractile globules which are so distinctive a feature in the majority of yeasts. It appears probable that these globules—by some observers regarded as spores—may result from the fusion of the smaller particles which at a certain stage of the cell-life are endowed with movement.

[1] *Zeitschrift für Hygiene und Infections Krankheiten*, Bd. xxi., p. 401.

This, however, I cannot, in the absence of actual observation, state as a fact. It is evident that the moving particles are hastened to their agglomerated and quiescent state by the action of the dye used, and it is very difficult to find a cell with motile particles without using some stain, inasmuch as the particles are extremely small and easily overlooked unless tinted by an anilin dye. These particles do not darken when kept in 1 per cent. solution of osmic acid for twenty-four hours, and they dissolve in a 2 per cent. solution of acetic acid; they are thus probably albuminous in nature. The fact that they fuse together differentiates them at once from the pigment particles which are described by Laveran[1] as often possessed of active movement in the body of the hæmatozoon of malaria. The phenomenon, moreover, differs from the movement of the plasma observed by Schionning[2] in a yeast in which he claims that ascospores are formed in a peculiar manner. Schionning observed the plasma of the yeast cell, which contained certain large and also small particles, move slowly as a whole, and finally settle upon the cell wall at various points. He makes merely a passing reference to this movement, and likewise states that the particles he observed are often united to one another by delicate "cordes de plasma."

Again there has been described by Fischer and Brebeck,[3] a peculiar phenomenon in a yeast-like organism—endoblastoderma pulverulentum—which is associated with a movement on the part of the contained corpuscle. There appears in the cell a small highly refractile body which gradually grows larger until it is 2 μ in diameter; then this body moves to the wall of the cell and gradually passes through the cell-wall and remains attached as a bud. It is evident that these phenomena are quite different from the one described in this paper, but all agree in the possession of movements which pass before the eye of the observer and claim attention and further study. In conclusion, reference may be made to

[1] "Paludisme," Laveran, 1892 (Collection Léauté), pp. 25, 28, 33.

[2] *Annales de Micrographie*, Nov., 1895, p. 193.

[3] *Centralblatt für Bakteriol- und Parasitenkunde*, Bd. xiv., 1893, No. 20. See also *Annales de Micrographie*, July-August, 1895, p. 320.

an interesting observation of Rosenthal.[1] This investigator, when examining the cells of perfectly fresh cancers, found certain intra-cellular bodies with double-contoured walls, within which were numerous minute particles which danced with very active movement. These moving particles he concludes are merely minute fat globules resulting from fatty degeneration of the included bodies, and their movement he considers to be molecular. As yet Rosenthal's observation is isolated, and in view of the recent work on the blastomycetic nature of the cancer parasite it is of interest to note that a somewhat similar phenomenon occurs in the cancer bodies and in numerous, perhaps all, yeast cells when these latter are examined in pure cultures.

[1] *Archiv für Gynakologie*, Bd. li., Heft 1.

ON MICROCOCCUS GONORRHŒÆ AND GONORRHŒAL INFECTION.

BY ALEX. G. R. FOULERTON, F.R.C.S.

Of all the recognised pathogenic bacteria there is probably none which has been the cause of wider diversity of opinion amongst those who have studied its natural history than the micrococcus which Neisser first described in 1879 as the cause of gonorrhœal urethritis.

Nor are the reasons for this diversity of opinion far to seek. First there is the fact that the lesions most frequently caused by micrococcus gonorrhœæ occur on what must, for bacteriological purposes, be regarded as exposed surfaces, and consequently become almost certainly infected within a very short time by other pathogenic bacteria as well. And so it often becomes most difficult either clinically or bacteriologically to differentiate the pathogenic action of the respective micro-organisms when one comes to consider the immediate and the remote sequences of gonorrhœal infection. Then again, there is the difficulty which has been experienced in finding an artificial soil suitable to the cultivation of the gonococcus. And finally there is the impossibility of controlling results by inoculation experiments on animals lower than man, since all such have hitherto proved immune against it.

The writer's own views on the purely bacteriological aspect of the question, as expressed in what follows, are founded on the results of an investigation with which he has been occupied during the last two years, and which has, in addition to other work, included the systematic examination by culture methods of the pus from fifty-eight different cases of acute gonorrhœal urethritis in the male.

I.—Morphology and General Characteristics.

The gonococcus of Neisser (*Micrococcus gonorrhœæ*) is a micro-organism of an irregularly oval shape which has been roughly compared with that of a coffee bean. It is arranged for the most part in pairs—as a diplococcus. The pairs have themselves a tendency to be arranged in couples, and so when a portion of a colony is lightly rubbed out on a cover-glass the diplococci are often seen to have a markedly tetrad grouping. In a pair the opposed surfaces are either almost flat or slightly concave, whilst the outer surface of each element of the pair is distinctly convex. The cocci multiply by fission, and the concavity on the surface of a coccus is an indication of the commencement of this process. The concave or flattened surface is also that surface which was formed by the line of fission in the immediately preceding generation. The lines of fission occur, therefore, in two directions of space; the line of fission in each generation taking a direction at right angles to the line of fission which occurred on the immediately preceding generation. And from this method of fission comes the tendency to a tetrad grouping frequently seen in the case of cultures grown on artificial media. In the small natural colony which occurs in a pus cell, on the other hand, this tendency to a tetrad grouping is not so noticeable, possibly because the mutual relations of the cell contents are altered during the process of "fixing" the specimen. But the number of cocci in such a colony will nearly always be found to be contained in the series 4, 8, 16, &c.

Individual cocci vary somewhat in size; the average measurements are probably about ·7 μ in the longer diameter and about ·5 μ in the shorter one. As to this, Sternberg says: "the diameter of an associated pair of cells varies from 0·8 to 1·6 μ in the long diameter—average about 1·25 μ—and from 0·6 to 0·8 in the line of the interspace between the biscuit-shaped elements."

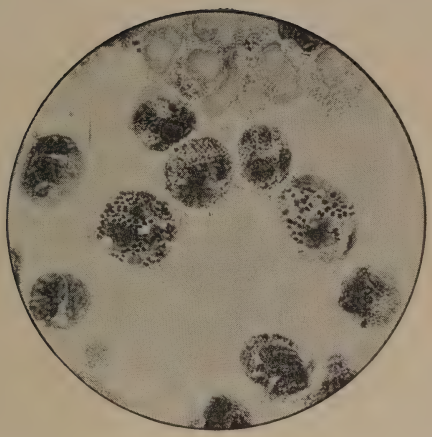
And further, it will sometimes be noticed that of a pair of cocci one is distinctly larger than the other; and also that of such the one which is of larger size will stain more deeply

with anilin dyes than will its fellow. And after staining and subsequent decolourisation by Gram's method it may be observed that the larger coccus of the pair parts rather less readily with the gentian violet stain than does the smaller one. This disparity, it should be added, I have observed only in the case of cocci after cultivation on an artificial soil.

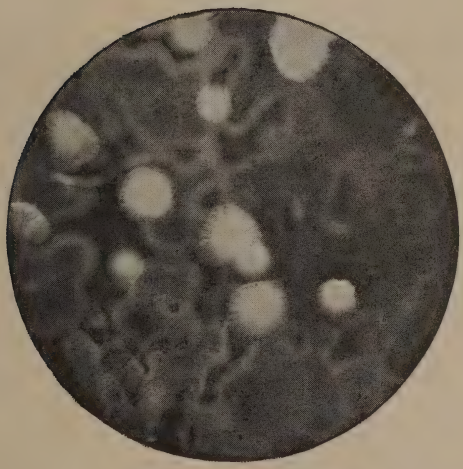
A capsule is said to envelope each pair of cocci. But although this is accepted as a general fact, I have not yet been able to demonstrate the structure to my own satisfaction in the case of cocci occurring in pus cells. In cultures grown on serum-agar, on the other hand, a definite capsule can often be made out. The gonococcus is said to possess a certain degree of inherent motility. Moore claims to have demonstrated this property of the cocci when contained in pus cells, but the matter cannot be looked upon at present as settled.

The coccus stains well with aqueous solutions of the ordinary anilin dyes. And of these Löffler's methylene blue and the methyl violet stain give, perhaps, the best results. It is decolourised when treated by Gram's method, after having been stained with anilin gentian violet.

The gonococcus is a typical parasite; its vitality is therefore depreciated by separation from its host. But at the same time it is probable that its power of independent vitality has been somewhat underrated in the past. I have myself obtained successful subcultures from colonies twelve days old, and Heiman obtained subcultures from colonies twenty-one days old, in which involution forms of the coccus were predominant. The specific virulence of the gonococcus, on the other hand, does not appear to be attenuated to any appreciable extent even after many generations of independent existence on artificial soils. Thus Bumm produced a typical acute gonorrhœal urethritis by inoculating a man's urethra with a culture removed by twenty generations from the original pus. Inoculations on the human subject carried out on the continent have shown that subcultures from the discharge in old cases of chronic urethritis of gonorrhœal origin will, when inoculated into a healthy urethra, set up a typical acute gonorrhœal urethritis. Another fact which



Micrococcus gonorrhœæ in pus cells × 1000.



Colonies of micrococcus gonorrhœæ. Seven days' growth on blood smear on agar plate × about 30 (?).

seems to have been brought out by these experiments is, that such a subculture from the discharge of a case of chronic urethritis, if re-inoculated into the same urethra from which the pus was taken, will not set up any fresh disturbance; but that inoculation of the same urethra with cocci obtained from another case of gonorrhœal urethritis will, on the contrary, set up a fresh acute attack.

The gonococcus as a parasite attaches itself only to man. No reliable inoculation experiments on the lower animals have been recorded as successful up to the present time.

The specific virulence of gonorrhœal pus is destroyed by exposure to a temperature of 60° C. for ten minutes (Sternberg).

Cultivation of the Gonococcus on Artificial Soils.—The gonococcus will grow on a suitable artificial soil at any temperature between 25° C. and 38° C. Growth at the lower extreme is very scanty; the optimum temperature is probably between 35° C. and 37° C., whilst the higher limit of temperature for growth is very sharply defined. Desiccation of the growth destroys its vitality very rapidly; immersion in water destroys it in a few hours. On a suitable soil the coccus will grow freely enough in the presence of oxygen; whether or not it will grow under absolutely anaerobic conditions is at present a matter of some doubt. The gonococcus will grow well when the reaction of the artificial soil is either feebly acid or feebly alkaline, but a high degree of acidity is less injurious than a corresponding degree of alkalinity. Nothing is known of the chemical products of the gonococcus, whether when growing in the body or on artificial media.

It is not proposed to discuss at any great length the various artificial soils upon which the gonococcus will grow, or upon which it is said to have grown. The very number of these is eloquent as to the difficulty which has been experienced in finding a suitable and at the same time readily available medium for the purpose. Some of those media will be first mentioned which I have myself tested without any success, and it may be added that in the case of each medium many carefully repeated experiments have

been made. Thus, the urine peptone broth of Finger has failed completely in my hands. Nor has Turro's method of culture on acid gelatin given any better results. As to this last, indeed, I entirely agree with the conclusion at which Heiman, of New York, arrived. It seems to be practically certain that the coccus which Turro described as flourishing on this medium was not the gonococcus at all. It appears to have been a larger coccus which grows well on acid, and for the matter of that equally well on alkaline gelatin, and retains deeply the gentian violet stain after treatment by Gram's method. This coccus, which may be conveniently named diplococcus urethræ communis, will be referred to again.

In like manner trials with Redner's saline peptone agar, and with Finger's urine agar, have proved equally sterile of result. By smearing pus over the surface of these last two media I have certainly succeeded once or twice in getting the growth of a few colonies. But more than this is necessary in order that a soil may be accounted fruitful; for, as Neisser has pointed out, it is easy enough when inoculating a solid medium with pus to carry on the inoculating instrument quite enough albuminous material along with the cocci for their subsequent nourishment, irrespective of the medium upon which they are implanted. This explains the difficulty which has confronted some workers, who have found that whilst their particular medium would grow the gonococcus direct from pus, yet a similar soil could not be used for the transplantation of the next subculture; and, therefore, no soil can be considered as a suitable one until it has proved capable of bearing a transplantation of the coccus under conditions which will exclude any trace of the original pus. The method of culture upon plover's egg-albumin as devised by Schrötter has not been tested.

Wertheim's blood-serum agar is the soil which has in the past been most frequently and most successfully used for the culture of the gonococcus. The addition to the medium of sodic phosphate as suggested by Ghon and Schlagenhaufer, or of glucose or glycerin as suggested by

Micrococcus gonorrhœæ × 1000.

Diplococcus urethræ communis × 1000.

Kral, does not seem at all necessary. For blood-serum, however, other serous fluids may conveniently be substituted. Thus, Steinschneider used hydrocele-fluid agar, but not apparently with very constant results. Kiefer found in ascitic-fluid agar a satisfactory medium. Heiman has used chest-serum agar with marked success, obtaining the fluid from cases of pleuritis. J. Homer Wright, of Boston, U.S.A., has grown the coccus on a mixture of urine, serum and agar, obtaining the serum indifferently from human or bovine blood. I have tried this medium, with the substitution of blood from the horse, but not with particularly good results. Menge used ovarian-fluid agar, a medium which I have also found useful. Aufuso succeeded with a combination of agar and the fluid obtained from a case of gonorrhœal arthritis, a result again which I am able to confirm from experience. Blood-serum from the rabbit, sheep, ox and dog, has also been used at different times, but with varying results—sometimes successfully, sometimes not. I have tried Löffler's horse serum for the pus of eight different cases of gonorrhœal urethritis, but with failure in every case. Stokes, of Boston, has, according to J. Homer Wright, succeeded in growing the coccus on this medium after it had been rendered slightly acid by means of hydrochloric acid. A fluid medium has been used consisting of one part of sterile human serum, with two parts of peptone beef broth. Albuminous urine from cases of nephritis will serve the same purpose.

The different serum agars referred to may be conveniently prepared and used in the following way. The serous fluid diluted, if very thick, with a little fresh urine is sterilised by filtration through a Berkefeld filter. In carrying this out considerable *vis a fronte* must be supplied by either an air or a water pump. The agar is prepared and sterilised in test tubes in the ordinary way, melted in a water bath, and cooled down to 40° C. The sterile serum is then added to the fluid agar in the test tube, which is then sloped; or the mixture is poured out into a Petri dish if plate cultures are desired. Before using the serum the flask containing it is kept for some little time in a water

bath at blood heat. And, since the number of contaminating organisms present in gonorrhœal pus from the primary lesion in either sex is usually considerable, plate cultures are in the majority of cases absolutely essential when endeavouring to isolate the gonococcus from discharges. The plates are inoculated by very lightly smearing the pus over their surface by means of a small sterilised camel's hair brush. Diluted plate cultures are obtained by passing lightly over a second and a third plate the same brush which has already been used for the first. As an alternative method the following may be used. Three tubes are filled, each with the quantity of sterile serum required for one plate. The first tube is inoculated with the pus by means of a platinum wire in the usual way, the second tube is inoculated with a loopful or two from the first, and the third in like manner from the second. The serum in each tube is then added to about twice its volume of fluid agar at 40° C., and the mixtures are poured out into three plate dishes in succession.

The quantity of serous fluid which must be added to the agar varies with the amount of albumin present in the former. Wertheim originally used one part of serum, obtained from human placental blood, with two parts of agar, and about this proportion will be found to be necessary in the case of the thinner serous fluids; but with blood serum itself a rather smaller proportion, say one part to three by volume, will give satisfactory results. If the serum is inoculated before mixing with the agar, care must be taken that the temperature of the latter does not exceed 40° C., since there is the possibility of injuring the vitality of the coccus by any higher temperature. If the plates are prepared before inoculation the mixing of the media should still be carried out at the same temperature. For although none of the serum proteids become visibly changed at temperatures below 60° C., there is reason to believe that their nutrient value for pathogenic bacteria may be considerably altered by an amount of heat which is not sufficient to cause a turbidity of the fluid which holds them in solution. It is, therefore, safer in any case to prepare all the serum

agars at the lowest temperature practicable; and this temperature is 39° to 40° C. And also for this reason I consider the method of sterilisation by filtration to be preferable to that of preparing serum media by fractional sterilisation at 60° C. for several successive days.

In addition to these serum agars I have, however, obtained some success with an egg-albumin mixture. The medium is prepared by mixing two parts of fluid agar at 40° C. with one part of human urine, to which egg-albumin in the proportion of 5 per cent. by volume has been added. The urine used has been of a normal acidity, that is to say of an acidity equivalent to between three and five parts per thousand of oxalic acid, and has been sterilised by filtration through a Berkefeld filter after the addition of the albumin. This filtration, owing to the tenacious character of the urine mixture, takes place very slowly and only under the influence of a considerable pressure. It is probable, however, that the proportion of egg-albumin might be reduced still further. I have not as yet made sufficient trial of this medium to enable me to report definitely upon it, but good growth of the gonococcus has been obtained by transplanting to it pure cultures from serum agar. As a matter of fact there is no reason to suppose that it will give any better results than those obtained by using a similar mixture of agar with albuminous urine from cases of nephritis; but the egg-albumin mixture had been used some time before the latter was suggested.

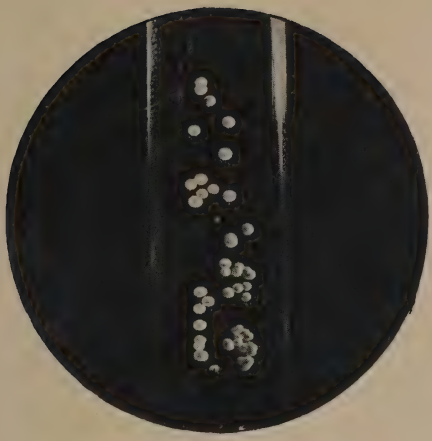
Another method which I have used extensively, and which is admirably adapted for general clinical work, is that of cultivation on a smear of fresh human blood, as first suggested by Abel. In detail the following is the plan of procedure which I have adopted. Three small Petri dishes, of about one inch and a half diameter, are prepared as ordinary agar plates. A small drop of fresh blood, obtained by pricking the finger, is then deposited from a sterilised capillary pipette upon the centre of each plate. The smallest possible quantity of pus, if pus is to be inoculated, is then blown on to a small sterilised camel's hair brush from the capillary tube in which it has

been collected. This pus, together with the drop of blood already deposited on the agar, is lightly rubbed out over the surface of the first plate. The blood drop on the second plate is then rubbed out with the same brush, and so the blood on the third.

The blood for the plates is collected after washing the finger, first with soap and water, then with a 5 per cent. solution of carbolic acid, and finally dipping it into alcohol which is allowed to run off. The finger is pricked, and a drop of blood allowed to collect. The point of a fine pipette is then dipped into the blood drop, from which a small quantity runs up the tube under the influence of capillary attraction. A separate pipette is used for each plate, and in practice no difficulty has been found in avoiding extraneous contamination of the preparations. The small amount of blood pigment on the plates does not interfere in any way with the recognition of the colonies.

The results, then, of my own experiments may be summed up as follows :—The gonococcus will not grow on any of the ordinary media of the laboratory. With the single exception of the egg-albumin mixture still under trial, I have found that the gonococcus will not grow satisfactorily upon any one of the special media which have been used unless that medium contains as a constituent a certain proportion of human serum-albumin. And in this my experiments have been quite in accord with those carried out by Neisser and others.

Growth of the Colonies on Artificial Media.—In plate cultures the growth of the colonies of gonococci either on serum-agar or on a blood smear on agar occurs as follows :— At the end of from twenty to twenty-four hours a number of small, slightly raised, transparent, greyish specks will be seen on the surface of the medium; viewed from the under surface of the plate and by transmitted light, these colonies have been well described as having the appearance of minute drops of dew. Up to the end of seventy-two to eighty hours the colonies increase in size and become slightly more prominent. After that time no increase in size will be evident. Examined with a low power of the microscope at



Colonies of diplococcus urethræ communis. Seventy-two hours' growth on gelatin, natural size.

the end of twenty-four hours the colonies are seen to be round in shape and with a fairly even and definite margin, finely granular in appearance, and of a faint brownish colour which is rather deeper towards the centre. At the end of about seventy-two hours the diameter of a colony will be about two millimetres or less. The colony will then be irregularly roundish in shape, with an uneven margin. In each colony there is a circular central region more opaque than the rest. The depth of colour shades off from the central portion to the periphery, and there is an outer zone which varies somewhat in extent with the age of the colony and which is devoid of definite colour. The central most opaque portion is usually distinctly circular, even if the colony as a whole is more or less irregular in shape. The whole colony with the exception of the outermost colourless zone is decidedly granular. And in the central portion of the colony distinct collections of larger granules are usually to be seen, which appear of a darker brown and are slightly more refractile than the rest.

The consistence of the colony is tenacious and slimy. Using a low power of the microscope and manipulating the colony with a platinum wire, it will be seen that the colony has very little tendency to adhere to the point of the wire. If an attempt is made to lift the colony up on the point of the wire it at once slips off again in a jelly-fish-like manner. In older colonies this tendency is to a certain degree lost.

If the plates are kept in the incubator at 37° C. without any precautions against loss of moisture, the appearance of a colony will by the end of a week or so be somewhat altered. The growth will have a somewhat crinkled appearance, and a number of fine lines will be seen running from the margin of the colony towards the centre. The colony will be of a darker brown tint than the younger colony. The colourless peripheral zone will be sharply defined, and the margin of the colony will now be very definite and marked by a distinct brownish edge. These later changes are not, however, nearly so distinct if the plates have been kept in a moist chamber whilst incubating.

The Identification of the Gonococcus for Clinical Purposes.—One has now to consider the identification of the gonococcus under the various circumstances in which it may occur as a parasite of man; and the following are the chief points which must be taken into consideration:—(1) The result of cultivation experiments on artificial media; (2) the relation of the cocci to pus cells and various tissues; (3) the general appearance and staining reactions of the cocci. Inoculation experiments on animals are not, as already mentioned, available in the case of this disease. In a general way the gonococcus will have to be sought for during the life of the patient in fluids only, that is to say, in pus and in the exudations from synovial or serous membranes. Should death occur one may have to examine certain tissues, such as the wall of the urethra, the valves of the heart, or cartilage from joints.

For practical clinical purposes one has most frequently to examine pus derived from the primary seat of the gonorrhœal infection, from the urethra of the male, from the urethra, cervical canal and vagina of the female, and from the conjunctiva. Such pus is almost always contaminated by other bacteria, but still it should be collected for examination under conditions which will diminish the amount of contamination as much as possible. In the case of the urethral infection in either sex the anterior end of the canal is emptied of pus by gentle pressure, applied in the case of the female by the finger on the vagina. The pus from the deeper portion of the urethra is then pressed forward in the same way and collected in a sterilised capillary glass tube, the end of which is carefully inserted a short distance within the meatus. A specimen of pus from the cervical canal may be collected either on a camel's hair brush which has been sterilised in a plugged glass tube, or by means of a capillary tube passed up through the speculum by forceps. Pus from the vagina is similarly collected, but care must be taken to avoid mixture of the pus with the lubricant used for the speculum. If the pus becomes thus contaminated with fatty matter, and another specimen cannot be obtained, the difficulty can be got over, so far as

cover-glass specimens for staining are concerned, by drying the pus on the cover-glass in the usual way, and then soaking it for a quarter of an hour in ether.

In carrying out cultivation experiments for diagnostic purposes it is essential that plate cultures should be made; and I prefer above all others the method of culture on a smear of fresh human blood carried out as already described. It has seemed to me that on this medium during the first twenty-four hours there is a distinct tendency for the gonococcus to outgrow the other bacteria which are more frequently associated with it. Thus a plate which after twenty-four hours' incubation at 36° C. will show to the naked eye only colonies of the gonococcus, may at the end of forty-eight hours show a preponderating growth of other micro-organisms.

Three plates should be made, and it is as well to incubate one of them at 20° C. for purposes of comparison. Sub-cultures should be made from the plate colonies and incubated at the same temperatures. These sub-cultures will most conveniently be made on sloping serum agar tubes. Control tubes of glycerin agar and gelatin should also be inoculated from the colonies, and incubated at 36° C. and 20° C. respectively. Cover-glass specimens made from the subcultures on serum agar are then examined in order to complete the diagnosis. These cover-glass specimens will include one which has been stained with anilin gentian violet, decolourised by Gram's method, and finally stained with Bismarck brown.

The bacteriology of the contaminating micro-organisms in gonorrhœal pus is a large subject. The organisms met with are more frequently micrococci than bacilli, and the former are also the more important in that they may be possible sources of error in diagnosis.

The series of fifty-eight cases of gonorrhœal urethritis in the male which I have investigated were all picked cases with acute symptoms, none of which had previously been under treatment of any kind. One or two of the cases in which the discharge was of more than a week's duration were included, because the symptoms were still from a clinical point of view acute.

The following table gives the length of time which the discharge had existed when the pus was taken for examination, when that time could be ascertained from the patient. In those cases in which the time is not given the patient was uncertain as to this point, but in each such case the discharge was probably of not more than one week's duration.

In the first case on the tables the patient was examined within twenty-four hours of infection, in the remainder the date has reference to the first appearance of a discharge.

	Cases.		Cases.
Within twenty-four hours ..	1	Not stated, but probably within one week	6
First day	3	Tenth day	1
Second day	5	Fourteenth day	2
Third day	9	Twentieth day	1
Fourth day	11	Twenty-second day	1
Fifth day	9	Fifth week	1
Sixth day	3		—
Seventh day	5		58

The reaction on litmus paper of the discharge from the urethra was ascertained in twenty-seven cases.

	Alkaline.	Acid.	Neutral.
Within twenty-four hours	0	0	1
First day	1	1	0
Second day	1	0	0
Third day	2	0	0
Fourth day	5	1	1
Fifth day	3	1	0
Sixth day	3	0	0
Seventh day	3	1	0
Fourteenth day	0	1	0
Twentieth day	1	0	0
Twenty-second day	0	1	0
	—	—	—
	19	6	2

In fifty-seven cases out of the fifty-eight investigated, the gonococcus was identified either by cultivation methods, or in stained specimens of the discharge, or in both ways. In the remaining case the specific coccus could not be found.

In two cases only was the gonococcus the one micro-organism which grew on the plates. One of these was the case in which a discharge was present and examined within twenty-four hours of infection, and the other was a case in which the discharge was of five days' duration. I do not think, however, that with a larger series of cases even two cases out of fifty-eight would give pure cultures.

The results which I obtained do not confirm the statement sometimes made, that in cases of gonorrhœal urethritis the gonococcus is frequently the only micro-organism present during the first days of the discharge. Thus in each of the three cases in which the discharge was examined within twenty-four hours of the time when the patient first noticed its appearance, other bacteria in addition to the gonococcus were present. In one of these the staphylococcus pyogenes aureus and diplococcus urethræ communis were isolated. In a second case the staphylococcus pyogenes aureus was the only other micro-organism identified, whilst in the third case the same staphylococcus and a short fine bacillus grew on the plates.

The bacillary forms which occurred on the plate-cultures were not investigated with any care, but bacillus pyocyaneus was found on one occasion, and bacillus liquefaciens fluorescens twice. Bacillus coli communis was not noticed in any case.

Legrain mentions the following bacillary forms which he has found in gonorrhœal pus:—Bacillus No. 1, resembling bacillus Zopfii; Bacillus No. 2, growing in "luxuriant" white colonies on agar, but of a brown colour when growing on potato; Bacillus No. 3, a very short bacterium which grows on artificial media with difficulty.

The smegma bacillus might also be found, and Legrain isolated spirillum roseum in two cases. Of cocci other than micrococcus gonorrhœæ the two most frequently present were staphylococcus pyogenes aureus and that micro-organism which I have termed diplococcus urethræ communis. Streptococcus pyogenes was isolated in one case only of the series, and then occurred associated with micrococcus gonorrhœæ. Staphylococcus pyogenes citreus and staphylococcus cereus flavus were each isolated on one occasion only; and each time both micrococcus gonorrhœæ and staphylococcus pyogenes aureus were also present. In addition to the cocci with recognised pyogenic properties one must mention diplococcus subflavus described by Bumm and Lustgarten, and credited by the former with distinct pyogenic properties. Legrain, however, failed to obtain evidence that this coccus was in any way pathogenic.

A considerable number of other species of cocci have been described as existing in the healthy urethra and in pathological urethral discharges in the male. So far as one knows at present these cocci are all saprophytes; the only interest attaching to them is because of the possible error in diagnosis to which they might give rise. In a considerable number of my cases I isolated a large diplococcus which seems to have escaped the notice of those who have worked at the urethral bacteria, at any rate it does not correspond with any of the hitherto described species. For the purpose of distinguishing it from others it is here called diplococcus urethræ communis. It probably occurs in urethral pus as isolated pairs of cocci, and is found free in the pus serum, never in the cells. It grows on gelatin plates as elevated, opaque white colonies. On a gelatin smear it appears as similar large colonies, corresponding with the "billiard-ball" colonies which Turro described the gonococcus as forming on acid gelatin. Gelatin stabs show at the end of forty-eight hours, or sooner, a beaded growth along the line of inoculation. A gelatin streak preparation results in a dull white, dry-looking, beaded growth. A streak on agar results in a smooth greyish-white looking growth. Growth is rather more rapid on agar at 37° C. than on gelatin or agar at 20° C., and is quite obvious under the former conditions at the end of twenty-four hours. A free growth occurs on all the ordinary media. The cocci stain deeply by Gram's method, and no liquefaction of gelatin occurs at any time. The surface colonies on gelatin, when viewed through a low power and by transmitted light, are circular in outline, distinctly and uniformly granular, and of a darkish brown colour which is rather deeper at the centre than at the periphery. The margin is definite and regular. Colonies in the depth of the gelatin are of a uniform dark brown colour. Since I first isolated this coccus a somewhat similar one has been described by Heiman, of New York. Heiman believes that the coccus which he describes is the one with which Turro was working when he devised the method of culture on acid gelatin for the gonococcus. Heiman's description, however, differs from mine in some particulars

—notably as to its size. Heiman speaks of the coccus which he describes as corresponding in size with the gonococcus, whereas diplococcus urethræ communis is considerably the larger of the two.

Another diplococcus which I have isolated from urethral discharges is a small coccus closely resembling the gonococcus, both in its size and in a marked tendency to a tetrad grouping. It grows in round lemon-coloured colonies on agar and gelatin plates, the latter not being liquefied. Like the gonococcus it is very readily decolourised when treated by Gram's method. In the table on p. 56 it appears as diplococcus urethræ citreus.

The following table gives in brief the distinguishing features of the various cocci which may be met with in the secretion of the healthy urethra, or in pathological urethral discharges in the male. Several of them have also been isolated from vaginal mucus in the female. The ordinary pyogenic cocci have not been included in the list.

All these eighteen different species of cocci, with the exception of the last on the list which grows only on agar, differ, it will be noticed, from micrococcus gonorrhœæ in one respect—that is to say, they are capable of growth on both gelatin and agar. Therefore, when making sub-cultures from plate colonies for purposes of diagnosis one should never omit the inoculation of ordinary gelatin and agar tubes to be used as controls. If the coccus isolated should grow on agar at 36° C., or on gelatin at 20° C., it is not micrococcus gonorrhœæ.

With regard to the isolation of the gonococcus in cases of gonorrhœa in women, recent work has confirmed Steinschneider's observation that the specific coccus will be found most frequently in a urethral or vulval discharge, less often in a discharge from the cervical canal, rarely or never in the purely vaginal discharge. I have never myself been able to identify the gonococcus in a stained cover-glass specimen of vaginal pus, nor to isolate it by cultivation methods from such material. This point can of course be investigated only in such cases as show no evidence of infection of the cervical canal. One must here mention

	Name.	Described by.	Reaction with Gram's method of staining.	Growth on Gelatin.	
1	Orchiococcus	Hougonenq and Éraud	— Gram	Liquefying ..	Growth on agar. Described by authors named as cause of certain cases of orchitis.
2	(Diplococcus)	Steinschneider ..	— Gram	Liquefying ..	Yellow growth on agar.
3	Diplococcus albicans amplus	Bumm	— Gram	Liquefying ..	White growth on agar.
4	Large diplococcus	Heiman	+ Gram	Liquefying ..	Yellowish growth on agar.
5	Diplococcus subflavus ..	Bumm	+ Gram	Liquefying ..	Yellow growth on agar.
6	Diplococcus citreus conglomeratus	Bumm	+ Gram	Liquefies slowly ..	Lemon-coloured growth on agar.
7	Diplocoque blanc jaunâtre de l'urethre	Legrain	+ Gram	Liquefying ..	Yellow growth on agar. Rapidly loses its power of liquefying gelatin when grown on artificial soils.
8	Microcoque orangé de l'urethre	Legrain	+ Gram	Liquefying ..	Yellow growth on agar. Closely resembles staphylococcus pyogenes aureus, but liquefies gelatin more rapidly, and inoculation experiments give negative results.
9	Microcoque blanc à colonies foliacées	Legrain	+ Gram ..	Liquefying ..	Occurs in groups and short chains. White growth on agar. "Il forme, sur plaques de gelatine des très élégantes colonies composées de bandes concentriques, bordées elles-mêmes de prolongements foliacés."
10	Diplococcus albicans tardissimus	Bumm	— Gram? ..	Non-liquefying ..	White growth on agar.
11	(Grey-white diplococcus) ..	Steinschneider ..	— Gram	Non-liquefying ..	Greyish-white growth on agar.
12	Diplococcus urethræ citreus	Foulerton ..	— Gram	Non-liquefying ..	Lemon-coloured growth on agar.
13	Microcoque blanc grisâtre de l'urethræ	Legrain	+ Gram	Non-liquefying ..	Grows feebly on gelatin. Large greyish-white colonies on agar, which often assume a rosy tint.
14	Diplococcus urethræ communis	Foulerton ..	+ Gram	Non-liquefying ..	Greyish-white growth on agar.
15	Diplocoque jaune non liquifiant de l'urethre	Legrain	+ Gram	Non-liquefying ..	Growth on agar and gelatin "d'un beau jaune de chrome."
16	Diplococcus lacteus faviformis	Bumm	+ Gram	Non-liquefying ..	
17	Micrococcus Ochroleucus ..	Prove	+ Gram	Non-liquefying ..	Occurs as isolated cocci, as diplococci or as short chains; evolves a sulphurous smell during growth; colonies show a yellow centre with a surrounding zone of white.
18	Streptococcus giganteus urethræ	Lustgarten ..	.. .. ..	.. .. ..	No growth on gelatin at 20° C. Colonies on agar transparent and iridescent.

what may be termed the "pseudo-gonococci" which have been described as occurring in the vaginal pus.

Heiman, of New York, whose work has previously been referred to, has devoted much attention to this matter. He has described a coccus which he designates diplococcus colpitis catarrhalis, and which he believes to be the same micro-organism which had been previously described by Fränkel and Bockhart. This diplococcus colpitis catarrhalis is about the size of the gonococcus, occurs like it in the pus cells, but is not decolourised when stained and treated by Gram's method. It grows on agar and gelatin at both blood and room heat, and was innocuous when inoculated into the male urethra. Heiman found the micro-organism in cases of slight muco-purulent discharges in young girls. Fränkel's diplococcus was isolated from the discharges of female children during an "epidemic" of vulvo-vaginitis which occurred in the Hamburg Hospital. The coccus appears to have been indistinguishable from micrococcus gonorrhœæ, but inoculation of the male urethra with the pus from these cases was followed by only a very mild urethritis. There appears to be, as a matter of fact, nothing in Fränkel's paper to indicate that the coccus in question was not the gonococcus itself; and gonorrhœal disease has certainly before now been spread amongst female children by the common use of infected towels.

Bockhart's diplococcus was isolated from cases of vaginitis. It was found in the cervical and vaginal secretions only when these were of alkaline reaction, never when they were acid. It occurred but rarely in the pus cells, and when it did so occur never formed large colonies such as the gonococcus does. It grew on agar at 30° to 32° C., and set up a urethritis of short duration when pure cultures of it were transplanted to the male urethra.

J. Homer Wright, who has also studied this question, isolated a coccus resembling the gonococcus from the discharge in a case of vaginitis in a child nine years of age. In this case, although it is particularly stated that there was "no history of contagion or stuprum," yet the clinical appearances given are those of a case of acute gonorrhœal "vulvo-vaginitis."

Pus from cases of pyosalpinx or the discharge in cases of gonorrhœal conjunctivitis will be best examined in the same way as that suggested for urethral pus. In these cases, however, the number of contaminating bacteria will probably not be so large. In conjunctivitis staphylococcus pyogenes aureus will be the associated coccus most frequently met, and in cases of pyosalpinx the same staphylococcus or streptococcus pyogenes may be found together with micrococcus gonorrhœæ.

Plates may be prepared from the fluid in cases of suspected gonorrhœal arthritis by mixing it with twice its volume of agar which has been melted and cooled down to 40° C. The fluid will be most conveniently obtained by tapping the joint by means of a sterilised syringe.

The isolation of the gonococcus from the urine may be attempted by inoculating serum-agar plates with the deposit obtained by centrifugalising the urine, but in view of the very large number of other bacteria usually present the isolation will probably be a matter of great difficulty.

The Recognition of the Gonococcus in Pus.—The recognition of the gonococcus in stained cover-glass specimens is often, it need scarcely be said, a matter of considerable practical importance. I believe myself that this recognition can in the large majority of cases be made a matter of absolute certainty by any one who has had some little experience. The grouping of the diplococci in the pus cells, their long oval shape when they occur in this situation, and the comparative rapidity with which they are decolourised when treated by Gram's method, are together sufficiently characteristic to enable one to give a definite opinion should the gonococcus be present. No opinion should be given if only isolated diplococci free from the pus cells are seen, even if they are decolourised by Gram's method. Nor should one be prepared to give an opinion unless a pus-cell colony containing at least four pairs of cocci has been identified. The cocci lie around the nucleus and sometimes appear to indent it, they very rarely penetrate it. Neisser and others, indeed, say that actual penetration of the nucleus never occurs, but from my own observation I think that there can be no

doubt that the cocci do occasionally lie actually within the nucleus. Thayer and Blumer have also described the cocci as lying within the nucleus, and add that in such cases they may be surrounded by a clear unstained zone. Whether this last appearance is due to vacuolation of the nucleus or merely to the capsule of the coccus is not certain. Very careful preparation and staining of the specimen is required when investigating this particular point. When seen in a pus cell the cocci appear of a longer oval shape than when specimens from cultures on artificial media are examined. The arrangement as diplococci is usually very obvious. Pus cells will sometimes be seen which appear to have been recently ruptured, and from which the cocci seem to be escaping. In the majority of cases I believe that this rupture of the cell is brought about during the fixing of the specimen; at any rate since I have devoted more care to this detail far fewer of these ruptured cells have been seen. Typical colonies of four or more pairs of cocci do not in my experience occur in the epithelial cells which one comes across in the pus of urethritis in the male. Isolated pairs of cocci, or sometimes two pairs, may be seen apparently lying on such a cell, but the gonococcus probably does not flourish actually within the epithelial cells.

For general purposes no staining reagent gives better results with gonorrhœal pus than Löffler's blue, in which a staining of about two minutes is required. For double staining Löffler's blue for two minutes and an aqueous solution of eosin for thirty seconds may be employed. Gram's method should also be used, and in its employment some constant routine must be observed. Roux originally advised the following application of Gram's method for gonorrhœal pus:—Stain cover-glass specimen, after fixing in usual manner in an anilin solution of methyl blue prepared by adding 10 drops of a saturated alcoholic solution of methyl blue to 5 cc. of an anilin solution made by shaking up together 3 cc. of anilin oil, 7 cc. of absolute alcohol, and 90 cc. of water. After the cover-glass has been in the stain for two to three minutes it is to be washed by a single dip in water, and then placed for two minutes in

Gram's iodine solution. It is then washed in absolute alcohol until no more stain is removed, dipped into water, and finally counter-stained for two minutes in a concentrated aqueous solution of eosin.

The method which I have used throughout my investigations both for pus specimens and for pure cultures of the coccus has been the following:—Fifteen minutes' staining in anilin gentian violet solution, then a single dip into water, then two minutes in Gram's iodine solution, decolourisation in absolute alcohol until the drainings on white filter paper are devoid of colour, and a final counter-staining for forty-five seconds in a saturated aqueous solution of Bismarck brown diluted with three times its volume of water.

When investigating the exact relations of the cocci to the cell-nucleus the following method has appeared to be the most suitable. The cover-glass specimen is stained for from thirty to forty-five seconds in the following mixture:—10 cc. of a 1 per cent. aqueous solution of dahlia violet, 30 cc. of a 1 per cent. aqueous solution of methyl green. The specimen is then washed in water, dried between folds of blotting paper, and mounted. Stained by this method the cocci appear red, and the cell nuclei of a blueish green tint.

The somewhat rough method which is sometimes employed of "fixing" the pus on the cover-glass by passing it through the flame three times should be avoided. The pus to be examined should be spread out between two cover-glasses which are dried in the air as quickly as possible. The "fixing" of the specimen is effected by placing it for fifteen minutes in a mixture of equal parts of absolute alcohol and ether. By this method the use of any heat is avoided, and the mutual arrangement of the cells and their contents is less likely to be disturbed.

It has been said that when examining gonorrhœal pus for the specific coccus no notice should be taken of diplococci unless they occur in definite colonies in the pus cells. A glance at the list given of cocci other than the micrococcus gonorrhœa which may be found at one time or another in urethral pus, will show that there are, at least,

five which are decolourised when treated by Gram's method. But none of these, so far as we know, occur in the pus cells; they occur rather as saprophytes in the pus serum.

With regard to certain other diplococci which have been described as occurring in pus cells a few words must be said.

First, and most important, perhaps, is diplococcus colpitis catarrhalis, which we may take to be identical with Bockhart's diplococcus. This, according to Heiman, is similar in size and general appearance to the gonococcus, and, like it, occurs in cell colonies. It is not, however, decolourised by Gram's method. Bockhart's inoculation experiments are not quite convincing, but still the possibility remains that this coccus may be an occasional cause of urethritis in the male. On the other hand, there is the fact that up to the present time no diplococcus, other than micrococcus gonorrhœæ, which forms pus-cell colonies and is decolourised by Gram's method, has been found in naturally-acquired cases of urethritis in the male.

Bumm, in eight cases of "puerperal cystitis," isolated from the urine a diplococcus which in its form, grouping, and relation to pus cells resembled the gonococcus. It was also found in the lochia. It was apparently decolourised when treated by Gram's method, although this point is not very clearly stated. It grew on agar in yellow colonies, very much like those of staphylococcus pyogenes aureus.

And, since cases of supposed gonorrhœal infection of the meningeal cavity surrounding the spinal cord have been recorded, one must also mention in this place the diplococcus intra-cellularis meningitidis. This coccus was found by Weichselbaum in the exudation in cases of cerebro-spinal meningitis. It occurs in the pus cells, and also free in the serous exudation. It would, however, be readily distinguished from the gonococcus by its growth on artificial media. Thus, whilst it will not grow on any medium at room temperature, it gives a free growth on agar at blood heat. It is, moreover, distinctly pathogenic

for mice, guinea-pigs, rabbits and dogs, and is decolourised when treated by Gram's method.

One may sum up briefly what has been said as to the identification of micrococcus gonorrhœæ thus: — If pure cultures of the micro-organism as well as stained cover-glass specimens of pus or other fluid can be obtained and examined, no difficulty whatever will be experienced. For no diplococcus other than micrococcus gonorrhœæ is known which presents all three of the following characteristics: occurrence in cell-colonies in pus, decolourisation when treated by Gram's method, and inability to grow on agar at both blood and room temperature.

If the opportunity of making cultivation experiments is not afforded, even then one may, in the large majority of gonorrhœal cases, found a certain diagnosis on the appearance of typical cell-colonies, the individual members of which are decolourised when treated by Gram's method. With reference to this it should be remembered that the "pseudo-gonococci" have only been described as occurring in vaginal discharges, in which it seems probable that the gonococcus itself very rarely occurs. Opinions have from time to time been brought forward which disagree with those here expressed as to the possibility of diagnosing with certainty the existence of gonorrhœal disease by means of the examination of stained specimens of the pus. E. v. Hibler is the latest of those thus dissenting, and his papers on the subject (*Centralbl. für Bacteriologie*, January 23 and February 5, 1896) may be consulted.

II.—Gonorrhœal Infection.

The specificity of micrococcus gonorrhœæ as a pathogenic bacterium is now a matter which has been proved beyond all doubt. Bumm was the first to show that it complied absolutely with all the conditions required by Koch's postulates, and the same truth has since then been repeatedly tested. One may, then, start by saying that gonorrhœal disease is invariably the result of infection by one specific micro-organism, micrococcus gonorrhœæ. And

it has been further proved beyond all question that the direct pathogenic action of the gonococcus is not necessarily confined to the seat of primary infection, but may be exerted against parts remote therefrom. And so whilst we assume that the malaise and feverishness which are so commonly experienced during the early stage of a gonorrhœa are merely due to the absorption of some toxin or morbid product elaborated at the local lesion, we also know that in certain cases the gonococcus itself is carried in the circulation to parts at a distance from the seat of the primary inoculation, and there establishes itself and exerts its specific pathogenic virulence.

According to the situation in which it grows the gonococcus may or may not show evidence of pyogenic action. As was noticed at the commencement of this paper, it is often difficult to define the actual pathogenic qualities of the gonococcus as it grows in the primary lesion, on account of the presence of one or more of the other pyogenic cocci which become associated with it. The gonococcus has, however, been found to be the only micro-organism present in some cases of pyosalpinx and in other abscesses. On the other hand it has been found in pure culture in the fluid obtained from cases of arthritis in which there were no signs of suppuration either at the time or subsequently.

When studying the general phenomena of gonorrhœal disease and the direct effect upon the tissues of an invasion by the gonococcus, one may conveniently divide the subject into three sections:—

(1) The primary infection.

(2) The spread of gonorrhœal disease by direct continuity of surface.

(3) The general systemic gonorrhœal infection.

(1) *The Primary Infection.*

Urethritis in the Male.—In the male the urethra is of course the commonest seat of infection and growth. And one may judge of the rapidity with which the gonococcus can increase when on a suitable soil, by observing the spread

of a gonorrhœal infection along the urethra from the seat of inoculation, which must of necessity be situated within a very short distance of the meatus.

It may be assumed, from the rather scanty direct evidence at our disposal, that the cocci make their way through some breach in the most superficial epithelial cells, which do not seem to be directly affected by them. They then grow along the intercellular spaces of the epithelial layers, invading both the deeper epithelial cells and the leucocytes in the sub-epithelial tissue. It seems probable, however, that these deeper epithelial cells are much less likely to be attacked than the leucocytes. That the leucocytes are attacked rather than attacking is, I think, indicated by the fact that active multiplication of the cocci occurs within them. It is difficult to believe that such an increase would occur were the leucocytes actively antagonistic to this particular coccus. In the majority of cases the cocci do not penetrate beyond the layer of plain muscular fibre which bounds the mucous membrane. From this invasion by the cocci there results an inflammatory serous infiltration of the sub-epithelial connective tissue, with a consequent more or less extensive catarrhal denudation of the epithelial cells.

By the time that this stage is reached the inflamed mucous surface has probably already become infected by one of the commoner pyogenic bacteria, and so it is impossible to say how far subsequent changes are due to the gonococcus alone.

In a certain limited number of cases the opportunity has occurred for an examination after death of the urethral lesion. Thus Finger, Ghon, and Schlagenhaufer have recorded cases in which the urethra was examined in the earlier stages of the disease. The principal changes found were loosening and desquamation of the epithelial cells, and serous infiltration of the sub-epithelial tissue, within the cells of which gonococci were found. Dinkler, on examination of a urethra in a rather later stage of the disease, found that tracts of the epithelial cells were necrosed, whilst the sub-epithelial tissue was in a condition of purulent infiltration, with thrombosis of some of its capillaries. Gonococci were

found in the superficial epithelial layers and in the purulent submucosa, which latter was in places entirely denuded of epithelium. Councilman, in the case of a patient who died five weeks after gonorrhœal infection, found the epithelial layer crowded with leucocytes in which gonococci were to be seen; the sub-epithelial connective tissue showed no change at all.

The changes found in the later and chronic stage of gonorrhœal urethritis have been studied more particularly by Finger and Audry. So far as the tissues are concerned these changes may be summed up as comprising the conversion of cylindrical into squamous epithelium, more or less extensive catarrhal desquamation of the epithelial cells, and small-celled infiltration of the sub-epithelial tissue. Finger found the gonococcus in the sub-epithelial tissue in one case only. Andry notes the presence of the cocci between the cells lining the follicles of the mucous membrane.

The result of *post-mortem* examination also confirms what has been deduced from clinical observation as to the portions of the urethra which suffer most severely in gonorrhœal disease, that is to say the alterations in the mucous membrane are most marked at three points, about the lacuna magna at the anterior end of the urethra, in the bulbous portion, and in the region of the prostate.

Peri-Urethral Abscess.—The gonococcus has occasionally been found to be present in the follicular or peri-urethral abscesses which occur more especially either close to the meatus or at the bulbous portion. In such abscesses the coccus is sometimes found in pure culture, but more commonly in the company of other pyogenic bacteria.

Gonorrhœal Prostatitis.—In common with the urethral follicles, or glands of Litré, the follicles of the prostate frequently become implicated in the course of a gonorrhœal urethritis. This inflammation may result in a general prostatitis, sometimes in the formation of an abscess. The pus from such an abscess may, as McCosh has pointed out, make its way into the peri-prostatic connective tissue, and finally burst through into the peritoneal cavity. Another result of prostatitis is thrombosis of the prostatic sinus. The soften-

ing and breaking down of a thrombus so formed is probably the most frequent cause of the very acute and fatal pyæmia which now and again occurs in the early stage of a gonorrhœal urethritis. This septic thrombosis is probably the result of a mixed infection. Brouardel has recorded a case of sudden death with pulmonary embolism resulting from the breaking down of one of these thrombi.

A question of importance in clinical practice is that as to the length of time during which the gonococcus may persist in the urethra after the original infection. No very satisfactory answer can be given to this at present; but in cases of gleet there can be no doubt that the coccus, although not to be found in the discharge as it ordinarily comes from the urethra, is often still present in some part of the urethra, and that connection with such a patient may be followed by infection. Thus, when from any cause apart from re-infection, a fresh attack of inflammation occurs in a case of chronic urethritis of gonorrhœal origin, it has frequently been observed that the cocci reappear in the pus-cells, although they may have been absent from the discharge for some time previously. It will be remembered that in cases of chronic urethritis the gonococcus has been seen lying between the cells lining the urethral follicles, and it is easy to understand how they might be liberated and appear in the pus-cells during the process which follows a fresh attack of inflammation, induced, for instance, by an injection of nitrate of silver solution. Further, gonococci may sometimes be found in the so-called "prostatic threads" which are passed at the end of the act of micturition, long after they have disappeared from any gleety discharge which may exist. In some cases also the presence of the cocci may be ascertained by practising "expression of the prostate." This proceeding is carried out as follows:—The patient almost, but not quite, empties his bladder, the prostate is manipulated by means of a finger introduced into the rectum, and finally the remainder of the urine is expelled. This residue will contain a considerable quantity of the prostatic secretion, in the cells of which cocci may be detected.

The importance of examining the prostatic secretion

before deciding whether a man who has suffered from gonorrhœal urethritis is still liable or not to convey infection is obvious. Should cocci still be present in the prostatic follicles, there is every probability that they will be liberated with the prostatic fluid during ejaculation by the compressing effect on the gland of the anterior portions of the levator ani muscle.

Urethritis in the Female.—As in the male so in the other sex the primary manifestation of gonorrhœal disease is most commonly a urethritis; and it is easy to understand how the region of the urethral meatus in women is for physical reasons very liable to become infected.

Endocervicitis. — After the urethra the canal of the cervix is the most frequent seat of primary infection; and it also seems probable that the secretion of the cervical canal is the most common source of infection for the male; for whilst urethritis in women is not usually of long duration, the cervical disease is very persistent, and also may persist without any obvious symptoms. Menge selected thirty cases of purulent cervical discharge which had originated from a gonorrhœal infection. The pus from twenty of the cases was implanted on ovarian-fluid agar, in the other ten cases alkaline agar was the medium used for cultivation. Out of the twenty cases in which a medium suitable to the gonococcus was used, colonies of the coccus were obtained in eleven. The microscopic examination of the pus from these twenty cases showed that the gonococcus was present in thirteen, and in these cases was apparently the only bacterium to be seen. No bacteria of any sort could be detected in four of the cases. The plates of alkaline agar—a medium upon which the gonococcus will not grow—remained sterile in eight of the ten cases for which they were used; and of the ten cases the gonococcus could be detected microscopically in the pus of six, and again was apparently the only bacterium present in each case. Menge at the same time examined the cervical secretion of twenty healthy women who were supposed to have never contracted gonorrhœa. For this series of cases ordinary cultivation media were used, and the plates prepared from sixteen of

them remained sterile. In the other four cases saprophytic bacteria, which had probably strayed from the vagina, were found. These researches are of some importance as showing that a gonococcus infection of the cervical canal is not very prone to become a mixed infection, and are in accord with what is known as to the infections of the endometrium and tubes which are also more usually due to a pure gonococcus infection.

Steinschneider also examined the cervical secretion of a number of women who were known to have contracted gonorrhœal disease but were apparently cured, and found the specific coccus still present in 47 per cent. of the cases. This persistence in the cervical canal may be accounted for partly by the difficulty of applying adequate direct treatment to the part, and partly by the large number of glandular follicles here present, which seem to afford a favourable habitat for the coccus. And both these conditions, it may be remarked, apply equally to the case of the prostatic urethra in the male; and in the female an emptying of the follicles also occurs during sexual excitement, caused, as Dembo observed, by a gradual contraction of the uterine musculature following on stimulation of the anterior and upper part of the vagina.

Vaginitis.—A primary gonorrhœal vaginitis seems to be a rare occurrence, if, indeed, it ever happens. Why this should be so is not by any means evident, but the fact remains that the gonococcus is very seldom seen in the pus of a case of vaginitis, even when that vaginitis accompanies a gonorrhœal urethritis. The relative frequency with which it is found in such cases is a matter as to which some dispute has arisen. Some deny that it is ever found, others find it in a certain number of cases. Menge identified the coccus in only five out of eighty cases of vaginitis. This being so, one has no other course to take than to assume that the vaginitis which does undoubtedly often occur in the course of gonorrhœal disease, is due to an infection by bacteria other than the gonococcus; and the micro-organism most often concerned in this is staphylococcus pyogenes aureus, which very frequently causes a secondary

infection of the urethral lesion of which the gonococcus was the primary cause.

This immunity of the vagina can hardly be due to the reaction of its secretion, as has been supposed by some, for in such cases the gonococcus has the opportunity at different times of availing itself of either an acid or an alkaline secretion. The normal secretion of the healthy vagina is acid, yet a primary gonorrhœal vaginitis, as has already been said, rarely if ever occurs. Given, on the other hand, a vaginitis occurring after a urethral infection, we still find that the coccus, if present at all, is present in only a very small proportion of the cases. Now, the contents of the vagina are in such cases markedly alkaline; for it has been clearly shown that infection by staphylococcus pyogenes aureus in particular will rapidly cause a change in the normally acid reaction. One would therefore expect that if an alkaline secretion were all that is required for its growth, the coccus would quickly infect the vagina from the urethral lesion after a soil was thus prepared for it. The only other explanation which offers itself, is that for some reason or other the thick stratified epithelial lining of the vagina is, like the external skin, not easily affected by the coccus. It has been said by some that this immunity of the vagina from gonorrhœal infection does not hold good in the case of young children. But, as a matter of fact, the so-called "vulvo-vaginitis" which occurs in young girls as the result of gonorrhœal infection by one means or another, would seem in the large majority to be really a "vulvo-urethritis," without any infection of the vagina above the hymen.

Inflammation of Glands of Bartholin.—In connection with the disease of the seat of primary inoculation in women one must just mention the inflammation of the glands of Bartholin, which is sometimes troublesome because of its persistence. These glands, the ducts of which open in close proximity to the urethra, not infrequently become infected; and bacteriological examination of the pus from such cases has shown that the gonococcus may be the only micro-organism which can be recognised

in or isolated from it. Gonorrhœal inflammation of Skene's tubes has been recorded in cases in which the terminations close to the urethra of the usually obsolete Gartner's ducts have remained patent.

Gonorrhœal Conjunctivitis.—Outside the genital region in either sex the conjunctiva is the surface most frequently infected. Here the changes following infection have been described by Bumm. In the gonorrhœal conjunctivitis of infants it was found that the cocci make their way along the intercellular spaces of the epithelial layer, and are soon to be seen in the underlying connective tissue. The consideration of the further changes which occur when the inflammation extends deeper than the conjunctiva need not detain us now.

Infection of Nose, Mouth and Skin.—Primary infection of other parts of the body occurs from time to time. Thus, infection of the oral and nasal mucous membranes is known to occur both in infants and in adults. In infants this infection usually occurs, as in the case of the conjunctival disease, during birth. And it is worth while to mention here that diplococcus meningitidis intra-cellularis, referred to in an earlier part of this paper, has occasionally been identified in the nasal mucus. This last coccus, then, having thus made its way from the meningeal cavity, might, if it were discovered in the nasal discharge of an infant, give rise to some confusion. A single case of infection of the external skin has been recorded by Lang, who found the gonococcus in the pus from an ulcer on the back of the hand. There is no reason to believe that venereal warts are ever due to the direct action of the coccus; but I have good grounds for thinking that some of the "soft sores" which occur on the penis are really of a gonorrhœal nature. The bacteriological evidence which I have of this is, at present, incomplete, and I would not suggest that such a causation is other than exceptional. The great majority of these ulcers are doubtless caused by the bacillus of Ducrey.

Proctitis.—The primary seat of gonorrhœal disease may, in very rare instances, be situated in the rectum. Disease of the rectum may also be caused by infection, by means of

pus derived from a genital lesion in the same patient being conveyed to the anus. This auto-infection may occur in either sex, but is obviously much more likely to occur in women. A possible sequence of it is a simple stricture of the rectum, a condition, it will be remembered, which is much more common in women than in men. I do not think, however, that a gonorrhœal proctitis has ever been verified bacteriologically.

(2) *Spread of Gonorrhœal Disease by Direct Continuity of Tissue.*

Having now considered the changes at the primary seat of gonorrhœal infection, one may proceed to trace the growth of the coccus along directly continuous surfaces to parts more or less remote. Thus, in the case of the male, an epididymitis may complicate an attack of gonorrhœa. In the female the inflammation may spread through the uterus and Fallopian tubes, and may, by this route, reach the peritoneum. In either sex an inflammation of the bladder, ureters, and pelves of the kidneys may occur as a sequence of gonorrhœal infection.

Epididymitis.—In the case of epididymitis there is very little evidence to be found as to the exact causation of the lesion, since the opportunity of accurately investigating the pathological changes in such cases is of extreme rarity. In one case Routier, on examining the pus from an abscess which developed in the epididymis during the acute stage of a gonorrhœal urethritis, found typical gonococci, which he clearly differentiated from the so-called orchiococcus. This last coccus, which was described by Hugounenq and Éraud as a cause of epididymis, is an organism which is said to closely resemble the gonococcus—differing from it principally in its cultural peculiarities. Bacteriological examination of the fluid from cases of hydrocele secondary to gonorrhœal epididymitis has hitherto always given a negative result.

Metritis.—With regard to the local extension of the disease in the female our knowledge is of somewhat fuller

measure. When referring to gonorrhœal endocervicitis it will be remembered that the coccus was found in the majority of the cases present in pure culture; and the same observation applies to the lesions situated above the cervix.

In the case of the uterus Wertheim has made some valuable observations. He found that while gonococci may be found in the mucosa, they are rarely to be detected on the muscular tissue itself. Menge records two cases of general metritis of gonorrhœal origin, in which there was marked glandular hyperplasia, with small-celled infiltration of the mucosa. In one case there was a patch of purulent softening in the substance of the uterus, and in the pus cells intra-cellular gonococci were found. Gonococci were also found in the epithelium. In general terms the more serious condition which may be caused by an infection of the uterus is an acute interstitial metritis, followed later by a state of the parts in which the glandular tissue of the endometrium is increased in bulk, and in which the connective tissue of the musculature may undergo considerable hyperplasia with more or less impairment of the muscular fibre itself. In less severe cases the condition remains from the beginning one of simple endometritis.

Salpingitis. — Gonorrhœal inflammation of the tubes generally comes under the notice of the surgeon in the form of a definite pyo-salpinx. The pus from such cases has frequently been examined, and the presence of the specific coccus proved. Wertheim, in seven cases of pyo-salpinx, found that the gonococcus was the only bacterium present in the pus. J. Homer Wright examined the pus from twenty cases of pyo-salpinx, both by microscopical and cultural methods. In four of the cases the gonococcus was isolated from plates inoculated with the pus. In fourteen of the cases the pus proved sterile when implanted on an appropriate culture medium, nor could the gonococcus be demonstrated by means of the microscope in any of them. In one of the two remaining cases there was a sinus opening on the skin of the abdomen, whilst the other case was of tubercular origin. Wright has collected 106 previously

recorded cases of salpingitis, in which the gonococcus has been sought, either with the microscope, or by cultural methods. He finds that in only thirty-four of these cases was the gonococcus found, that in sixty-one of the cases the pus proved free from any bacteria at all, and that in only eleven of the 106 cases were bacteria other than the gonococcus present. These figures do not include his own series of twenty cases examined, with a positive result in four. If these cases be included we have 126 cases examined, and the gonococcus found in thirty-eight, or in about 31 per cent. Bacteria other than the gonococcus were found in thirteen or in about 10 per cent. And in seventy-five, or about 60 per cent., no bacteria of any sort could be found.

Ovaritis.—Acute gonorrhœal inflammation of the ovaries, although sometimes spoken of in clinical practice, is not known as a definite lesion to the pathologist. Bacteriologically there is at present nothing to be said as to the chronic inflammatory changes in the ovary which are so frequently seen in connection with a pyo-salpinx, and are the result of the spread of the inflammatory process along the connective tissue of the meso-salpinx.

Brouardel, in addition to the case of thrombosis of the prostatic sinus in the male already mentioned, has recorded a case in which the breaking down of a thrombus in the veins of the broad ligament was followed by pulmonary embolism and death.

Peritonitis.—Gonorrhœal peritonitis in the female may possibly occur as the result of the escape of pus from a tube by the ostium abdominale into the peritoneum, or more commonly after the rupture of a tubal abscess. In any case the accident is not of common occurrence, and there are only one or two such cases recorded in which the gonococcus has been positively identified in the peritoneal pus.

Cystitis.—In either sex the bladder and upper part of the urinary tract may in like manner be affected by an extension of the disease along the mucous surface. But here, again, it is impossible to say in what proportion of the not uncommon cases of gonorrhœal cystitis, or of the far

rarer cases of gonorrhœal pyelo-nephritis, the gonococcus is itself the direct cause of the local trouble.

Bumm concludes that gonorrhœal cystitis is always the result of a mixed infection, the associated bacteria in such cases being staphylococcus pyogenes aureus, streptococcus pyogenes, or bacillus coli communis. Melchior, on the other hand, states that the gonococcus is sometimes the only micro-organism present. It is also said that the gonococcus will only be found in urine which is distinctly acid in reaction. Wertheim has obtained direct proof of the presence of the specific coccus in the mucous membrane of the viscus in a case of gonorrhœal cystitis. The case was that of a young girl who had been raped, and in whom the resulting " vulvo-vaginitis " was complicated by cystitis. An anæsthetic was administered, and a very small piece of the mucous membrane of the bladder was removed for examination. Wertheim found the gonococcus in the epithelial layers, and in the spaces of the sub-mucous tissue. He states also that he was able to distinctly see the cocci lying within the capillary blood-vessels of the connective tissue.

Cystitis appears to be a more frequent complication of gonorrhœa in the male than in the female sex, and in the latter the symptoms are usually less marked.

Pyelo-nephritis.—The bladder inflammation now and again extends to the kidneys, resulting in a pyelo-nephritis. Ureteral stricture is a somewhat rare pathological condition which has also been found once or twice in those who had previously suffered from gonorrhœal disease. In no case of pyelo-nephritis of gonorrhœal origin has the opportunity for *post-mortem* bacteriological examination at an acute stage of the disease occurred, and therefore we have no knowledge as to its exact pathology.

Infection of the Lymphatics and Glands.—There is at present some doubt as to whether or not the gonococcus is to be found in the inflamed lymphatic vessels of the penis in cases of urethritis, or in the inguinal glands. In cases in which these have suppurated, the coccus is said to have been identified in the pus cells. But further exact observa-

tions will have to be made before the point can be considered as settled.

(3) *General Gonorrhœal Infection.*

The manifestations of gonorrhœal disease which have just been considered are those which are due to an extension of the infective process from the seat of primary inoculation by means of a direct continuity of tissue, and independently of the passage of infecting cocci by the blood or lymph vessels. There remains to be considered the general infection of the system by the coccus after it has ceased to be merely a parasite of the mucous membranes.

The general fever which accompanies sometimes the onset of a urethritis is not, as already said, by any means to be considered as an indication of a general infection, but rather as the result of an intoxication with bacterial products derived from the local lesion.

Presence of Micrococcus Gonorrhœæ in the Blood.—In only one case, so far as I am aware, has an absolute proof of the presence of the specific coccus in the blood of the general circulation been obtained. In this case, recorded by Thayer and Blumer, death occurred as the result of a gonorrhœal endocarditis. During the life of the patient pure cultures of the coccus were obtained from the blood, and after death intra-cellular cocci were found in connection with ulcers in the mitral valve, and also in the urethral lesion.

The remote secondary lesions which may complicate what had hitherto been a strictly local form of gonorrhœal disease, and upon which one was for some time compelled to rely as the only evidence of the capability of the gonococcus to cause a general infection, are as follows:—various heart lesions, arthritis, pleuritis, teno-synovitis, various affections of the eye-ball, spinal meningitis, myelitis and periostitis. It is only, however, in the cases of the heart, joints, and pleuræ that we have positive evidence that the lesion is a direct effect of infection by the specific coccus. With regard to the other complications one can only describe

them as gonorrhœal in the clinical, rather than in the strictly pathological sense of the word. That is to say, they are known to occur, with varying frequency, in connection with gonorrhœal urethritis, but there is no pathological proof that they are dependent upon the actual presence of the specific coccus. For although in some of these doubtful lesions the presence of the gonococcus is said to have been demonstrated, the evidence of its presence cannot be accepted without further confirmation.

Gonorrhœal Arthritis.—It is in the case of joint affections more especially that the most convincing proof of the possibility of a general infection by the gonococcus has been obtained, apart from the actual finding of it in the general circulation. For if we can show that the specific coccus is actually present in an arthritic effusion, and that it can only have been derived from a urethral lesion, no further question as to the possibility of a general or blood infection can arise. Bordoni Uffreduzzi isolated the coccus from the synovial exudation of the knee-joint of a man who was suffering from a gonorrhœal urethritis : the cocci thus isolated were inoculated into the urethra of a healthy man, and there caused a typical gonorrhœa.

The most convenient method of examining a joint fluid for bacteriological purposes has already been described. The plate cultures obtained from such cases will vary in their character. The plates may, for instance, remain sterile of all bacteria. The gonococcus may not be present, but the plates may yield a growth of staphylococcus aureus, or albus, or streptococcus pyogenes. Or the gonococcus may be present along with any of the three other cocci mentioned. And in such a case we may assume that there was first an infection of the joint by the gonococcus, and that the site of the lesion thus caused proved a suitable resting-place for the other bacteria, which also were probably derived from the lesion in the urethra. And finally, the gonococcus may be the only bacterium present. In one case in which I examined the fluid obtained from the knee-joint of a man during the second week of an arthritis which had developed sixteen days after the first appearance of a gonorrhœal

urethritis this proved to be the case. The plates prepared from the fluid showed a pure culture of the specific coccus, and no other bacterium grew on them either at room or at blood heat. And this result is one which several bacteriologists had previously obtained. It may be mentioned that if such a fluid, or a pure culture of the coccus, is carefully introduced into a joint of one of the lower animals, only a transient synovitis results. Nothing very definite is known as to the exact location of the cocci in or about the joints of patients with gonorrhœal arthritis. But they certainly sometimes occur in the cells contained in the pathological synovial fluid, and intra-cellular forms are also said to have been found in the joint cartilages.

The arthritis occurring in the course of gonorrhœal disease may be mono-articular or poly-articular in its distribution. Of the larger joints the knee, the elbow and the wrist are said to be most frequently affected. I have also seen the ankle joint affected on several occasions. Affection of the smaller joints is rather uncommon, but the sterno-clavicular, the temporo-maxillary, those of the fingers and toes, those of the vertebræ, and even those of the larynx may occasionally suffer. Clinically, the joint affections which occur in connection with gonorrhœal urethritis may be most conveniently studied in the case of the knee. In this situation the earliest stage of the trouble may assume one of two forms. It may occur either as an acute primary synovitis with distension of the joint cavity, or as a peri-arthritis with secondary effusion into the joint. And I think that, as a rule, the cases which commence with peri-arthritic swelling, and in which the synovial effusion is a secondary result, are those of pure gonococcus infection, whilst the cases which from the very beginning show signs of synovitis are most frequently those which result from an infection by cocci other than the gonococcus. I would further suggest that in the cases resulting from a pure gonococcus infection the trouble is usually limited to an arthritis of one or two of the larger joints — and of these most frequently the knee — and usually terminates without suppuration, but

frequently with a certain degree of fibrous anchylosis which is often of the extra-capsular or false kind. And, on the other hand, that most of the cases of mixed or of streptococcus infection are poly-articular, are more likely to end in suppuration of one of the joints affected, and are those which are more dangerous so far as life is concerned. These various forms of joint complication are of more common occurrence in men than in women. Clinically they are all spoken of as "gonorrhœal," but it is only to those in which the gonococcus is actually present in or about the joint that the adjective in its pathological sense should be applied.

The incidence of arthritis in children who have become infected with gonorrhœal disease appears to be somewhat remarkable. French surgeons write of arthritis as complicating from 2 to 3 per cent. of all cases of gonorrhœal urethritis, vulvo-vaginitis, and conjunctivitis in infants and young children. Moncorvo records as many as fifteen cases of arthritis occurring amongst forty-seven cases of gonorrhœal disease in children, and says that female children suffer from this complication more frequently than do male. Balzer states that the mono-articular form is exceptional in children, and that when it does occur the knee is the joint most frequently affected, but that the commoner poly-articular form attacks the smaller joints, and chiefly those of the fingers and toes, and also that the prognosis in these poly-articular cases is more favourable than in the similar class of cases when occurring in adults. These statements, however, as to the frequency of joint affections after gonorrhœal conjunctivitis—which is by far the commonest gonorrhœal affection in children—must for the present be accepted with a certain amount of reserve. They would certainly hardly seem justified by the surgical experience of this country, and possibly the effective treatment of the primary lesion may have some influence on the frequency of the secondary joint affections.

Heart Lesions in Gonorrhœal Disease.—The heart complications which may occur during the course of a case of

gonorrhœa may assume the form of endocarditis, pericarditis, or myocarditis. The relative frequency of each of these lesions may be judged from the following table, which gives an analysis of the cases quoted by Barruol in his "Étude Clinique sur les Complications Cardiaques de la Blennorrhagie." This writer has collected a series of thirty-three cases on which heart complications supervened on a gonorrhœal urethritis. The cases include those recorded in French literature up to the end of the year 1895, and in addition to these many other similar cases have been recorded by English and German physicians.

	Men.			Women.			Total.	
	Cases.	Joints affected in.	Deaths.	Cases.	Joints affected in.	Deaths.	Cases.	Deaths.
Endocarditis	18	9	7	4	4	1	22	8
Pericarditis ..	9	6	3	1	1	1	10	4
Myocarditis ..	1	1	1	..	..	..	1	1
	28	16	11	5	5	2	33	13

These heart cases, it must again be clearly understood, were for the most part cases which had been diagnosed as gonorrhœal on clinical grounds; that is to say, they occurred in connection with an attack of gonorrhœal urethritis. In only a small minority of the cases was the diagnosis confirmed by bacteriological proof of the exact nature of the lesion. And it is probable that some of the cases were due to a streptococcus rather than to a gonococcus infection. Amongst the cases included in the above table is the one of Thayer and Blumer which has already been referred to. In this case gonococci were found enclosed in leucocytes which formed part of a thrombus which was attached to the mitral valve. His, Glukinski, Leyden and Michaelis have recorded other cases in which the cocci were found in thrombi on the valves in cases of endocarditis complicating a gonorrhœa. In these cases the mitral and aortic valves seem to be those which are usually affected. In Council-

man's case, which is also included in the above, purulent infiltration of the heart musculature was found. There were gonococci in pus cells between the muscular fibres, and a few in the epicardial membrane. The coccus could not be found in the pericardial fluid, but was identified in the pus from the urethra and also in the exudation of an affected knee-joint.

Pleuritis.—In certain cases of pleuritis occurring in young girls, and in connection with gonorrhœal infection, Bordoni-Uffreduzzi demonstrated the presence of the specific coccus in the pleural effusion. In another case, mentioned by Neisser, of gonorrhœal arthritis and double pleuritis occurring in a young girl, the cocci were also found.

Teno-Synovitis.—Cases of gonorrhœal teno-synovitis have been recorded, and in at least one such case the gonococcus was found by Howitz in the synovial effusion.

As to the other lesions which may occur with gonorrhœal disease, there is, because of a want of exact knowledge, very little to be said from a bacteriological point of view.

Gonorrhœal Eye Lesions.—Apart from the primary gonorrhœal conjunctivitis, which is caused by direct infection and has already been spoken of, certain eye lesions which seem to result from a systemic infection are occasionally met with. These eye affections most frequently assume the form of an iritis or an episcleritis, and more usually happen to those who have already had an attack of arthritis. Cases of gonorrhœal neuro-retinitis have been placed on record by Panas, Highet, and other French writers, but such complications must be very uncommon, and I do not think that they have been recognised in this country.

Other Complications.—As complications of rare occurrence one also finds periostitis, sciatica, and other forms of neuralgia or neuritis, myelitis, and cerebro-spinal meningitis mentioned. But beyond mentioning the fact that they have been recorded, one is not able to say anything more about these at present.

In concluding this paper I cannot omit the acknowledgment of my indebtedness to those who have assisted me very materially in my work. To Dr. Macfadyen, of the British

Institute, my thanks are especially due for help freely given. Mr. Edward Cotterell, Surgeon to the London Lock Hospital, has also been most kind in placing cases at my disposal for material. And finally to Mr. Barnard my acknowledgments must be given for the excellent photographs which accompany this.

NOTE.—It has not been thought necessary to add to the numerous existing catalogues of literature relating to the gonococcus.

Full bibliographies will be found in the following :—

(i.) A Clinical and Bacteriological Study of the Gonococcus-Neisser, by Henry Heiman, M.D. (*New York Medical Record*, June 22, 1895.)

(ii.) Article on Gonorrhœal Disease, by Neisser and Schäffer. (*Ergebnisse der Allgem. Ätiologie der Menschen—und Tierkrankheiten.* Part I., 1896.)

(iii.) Article on Gonorrhœa, by Balzer. "Traité de Médecine et de Thérapeutique," edited by Brouardel and others. Vol. II., 1896.

It should be added that the above paper was completed early this year, and, therefore, does not contain any reference to work published during the last twelve months. Amongst the more important recent papers on the subject may be mentioned:—

A Further Study of the Biology of the Gonococcus, by H. Heiman. (*Medical Record*, December 19, 1896.)

Contribution à l'Étude du Gonocoque et de sa Toxine, by J. de Christmas. (*Annales de l'Institute Pasteur*, No. 8, Aug. 25, 1897.)

THE STERILISATION OF MILK.

BY A. MACFADYEN, M.D. AND R. T. HEWLETT, M.D.

THE sterilisation of milk is carried out either with the view of increasing its keeping powers as a commercial article, or of rendering it incapable of communicating disease as a food. The proteids, carbo-hydrates and salts which milk contains, render it an admirable soil for the growth of a number of saprophytic and pathogenic bacteria. The most frequent and obvious evidence of this is the souring and curdling that occur after milk has been standing for some time. This change is due mainly to the action of the lactic bacteria, of which the bacillus acidi lactici of Hueppe may be taken as a type. These organisms set up a fermentation of the milk sugar, resulting in the production of free lactic acid, and a consequent precipitation of the casein, but they do not exert any dissolving action on the curd. Pure cultures of such organisms may serve the useful purpose of ripening cream in the process of butter-making. On the other hand, they may be responsible for abnormal fermentations, as for example occur in the manufacture of cheese. The cheese as a result, becomes riddled with holes through an excessive development of CO_2 and H_2 gases. Certain forms also produce an inflammation of the udder in cows. (Bac. Guillebeau A, B, and C.) The multiplication of the lactic organisms in milk takes place most rapidly at 25-30° C.

The curdling of milk may frequently occur without the production of acidity, through the formation of a rennet-like ferment by certain bacteria, *e.g.*, tyrothrix tenuis, &c.

A widely distributed group of organisms produce a pre-

cipitation and subsequent solution of the casein, accompanied by the development of butyric acid. The butyric acid fermentation proceeds rapidly at 30-35° C., and is mainly due to spore-bearing bacilli. They are therefore more resistent to heat than the lactic organisms, and present one of the chief obstacles to a complete sterilisation of milk. Whilst the Pasteurising process weeds out the non-sporing lactic bacteria from milk, a temperature of 100° C. for one hour is necessary to destroy the butyric acid organisms. And even when this has been accomplished, the milk cannot be called sterile. There still remain the spores of organisms belonging to the group of the hay bacilli which are able to resist a temperature of 100° C., for three to six hours. A sterilisation of milk to be absolute would consequently require six hours' heating at boiling point or an exposure for a shorter period to steam under pressure by means of the autoclave. These very resistent forms produce little visible change in the milk, but its taste becomes bitter and pungent. Certain members of this group have marked toxic properties, causing a fatal diarrhœa in young dogs and cats.[1] The optimum temperature for their growth lies above 24° C.

Occasionally pigmentations of milk occur through the action of chromogenic organisms (B. lactis cyanogenus, B. prodigiosus, &c.), and numerous other defects, such as "viscid milk," "bitter milk," are due to the agency of bacteria, of which our knowledge is still very imperfect. Dairy bacteriology now constitutes a special line of research, with which the names of Freudenreich, Weigmann, Conn, and others have become associated.

Milk may also carry the infection of diphtheria, cholera, typhoid and scarlet fevers from one person to another, as well as the tubercle bacilli from a diseased animal to the human subject. No method of milk preservation is therefore worthy of adoption which does not ensure a destruction of pathogenic organisms. A scrupulous attention to cleanliness in dairy operations is in itself capable of effecting a great deal. A casual examination of the dirt deposit to be

[1] Flügge, *Zeitschrift für Hygiene*, Bd. xvii., p. 272.

found in town milk is sufficient to convince any one of this. Freezing of the milk and the addition of preservatives are inadequate methods of procedure. The one efficient and trustworthy agent we possess is *heat*, and the only question is how is this to be best and most effectually applied. The main difficulty is that a complete sterilisation of milk cannot be attempted without running the risk of altering its physical and chemical properties. Browning of the milk occurs, and its taste becomes altered. The consumer naturally does not favour such an article, when fresh milk can be readily obtained at a smaller cost. Its commercial value is small, though it may at times be advantageously used for young children. For all general purposes an absolute sterilisation of milk is not necessary. The practical and hygienic requirements are adequately met by the process of Pasteurisation, if it is properly carried out, and the milk is subsequently cooled. The milk likewise retains its taste and aroma. Heat and cold are the agents to be jointly employed in the process. The temperature selected must be sufficiently high to be fatal to organisms producing a rapid decomposition of milk, but it must not exceed that at which the milk acquires a cooked flavour. The critical temperature, according to Duclaux, is 70° C., above this temperature milk rapidly loses its normal taste.

The Pasteurising process consists in heating the milk to 65° to 70° C. for a short time, and then cooling it rapidly to preserve the fresh flavour and to prevent an increase of the bacteria that remain alive. Two kinds of apparatus have been used for this purpose. In the one case the milk is allowed to flow slowly over a corrugated metal surface, which is heated externally by water or steam. The thin layer of milk becomes quickly heated and acquires the temperature of 70° C. for a short time, and then passes into a cooler. Thiel's apparatus is a type of this kind of arrangement. In the other case the milk is heated in bulk to the desired temperature by means of steam, it being at the same time constantly stirred to ensure a rapid and uniform heating of the whole fluid. It is then drawn off through a cooler. The apparatus of Ahlhorn, Reinsch, &c.,

illustrate this method of Pasteurisation. Machines of the above nature, when acting properly, will destroy about 70 per cent. of the saprophytic organisms, but the pathogenic forms are not killed with certainty. The exposure to heat is not sufficiently long, and the results vary. When the cooler and the receiving vessels are unsterilised the milk easily becomes recontaminated.

Bitter[1] has made an exhaustive inquiry into the best means of carrying out the Pasteurising process. The above methods were tested by him, and found to be unsatisfactory on the grounds indicated. The tubercle bacilli frequently remained alive, and a fresh infection of the milk readily occurred in the unsterilised cooler and cans, thus practically destroying the efficacy of the method. The first point to be considered was the construction of an improved apparatus, which would admit of the action of the desired temperature, *not* momentarily but for a given length of time. Further, the determination of the temperature that would produce an adequate disinfection and conservation of the milk, with the least alteration in its quality. And finally the exclusion of a possible reinfection after the milk has been Pasteurised.

The process will only commend itself for general use by fulfilling the following practical requirements :—

(1) The ability to heat the milk quickly and to retain it at the desired temperature, and whilst ensuring at the same time a uniform heating of the whole mass.

(2) The ability to Pasteurise large quantities of milk in a short time.

(3) Simplicity in the construction of the apparatus, so that the method can be readily and cheaply carried out.

Bitter, with a view of meeting these requirements, devised an apparatus of which the following are the cardinal points : The milk steriliser consists of a cylindrical vessel of tinned copper and is of about 50 litres capacity. A spiral of coiled tubing runs through it and opens externally at the top and bottom of the apparatus. The milk is warmed by a current of steam circulating through this system of tubes. A

[1] Bitter, *Zeitschrift für Hygiene*, Bd. viii., p. 240.

mechanical stirrer mixes the milk so that all portions become uniformly heated during sterilisation. The temperature is controlled by means of thermometers and when the process is finished the milk passes through a stopcock into the cooler. Bitter found that if the steam be turned off when the thermometer indicates 74° C., the whole of the milk becomes heated to 75° C. in 30 seconds. It was possible to keep the milk at this temperature for half-an-hour by opening the steamcock slightly from time to time. Other constant temperatures above or below this could easily be obtained, *e.g.*, 96° C. in 23-24 minutes and 68 C. in 10-12 minutes. The incrustations that usually occur in other forms of apparatus and interfere with their regulation did not arise to any extent and there was no difficulty in obtaining the desired heat.

The next practical point was to ensure sterility of the cooler and the cans. Pasteurised milk will keep twice as long in sterilised as in unsterilised vessels. The milk cans were accordingly steamed for 15 minutes. The cooler consists of a cylinder of tinned copper with double walls, and a stream of cold water circulates through coiled tubing in the outer mantle. The milk flows down the inner surface of the cylinder and becomes cooled on its passage to the reservoir below. The cooler is sterilized before use by steaming for 15 minutes.

Bitter found that pathogenic organisms were killed with certainty on heating the milk to 68° C. for 30 minutes in the above fashion. At the same time the appearance and taste of the milk were not altered. Under ordinary circumstances 68° C. for 20 minutes is sufficient. The majority of the fermentative organisms are destroyed by the process. The milk will keep for two days at 20° C. and three to four days at 15° C.

These experiments show that Pasteurisation of milk, if adequately carried out, will fulfil all hygienic and most commercial requirements. We undertook, in view of inquiries that had been made, an independent investigation as to the best means of carrying out the process of milk sterilisation. There is no doubt that Pasteurisation would

be more extensively employed if a trustworthy yet comparatively simple and inexpensive apparatus were obtainable.

There is only one really practicable method whereby milk can be rendered absolutely sterile and endowed with unlimited keeping powers, viz., the application of steam under pressure. And being convinced of this, our endeavour was to determine how the best results, short of complete sterility, might be obtained. Ordinary dairy milk was employed which is of course richer in organisms than milk obtained direct from the cow, and makes the test of any method more severe. The experiments are given exactly in the order in which they were carried out.

The first series was conducted to determine what results could be obtained with temperatures considerably under 70° C. For this purpose samples of milk were submitted in the first instance to a *fractional sterilisation*. The heat was applied on each of three successive days, and the number of organisms was estimated in a platinum loopful of the milk before and after sterilisation. The temperature was controlled by means of thermometers. The heat was applied for 30 minutes in each case.

Experiment A :—

Temperature, 55° C.

Fresh Milk	=	8250	bacteria.
Sterilisation 1 =		60	,,
,, 2 =		200	,,
,, 3 =		800	,,

Experiment B :—

Temperature, 60° C.

Fresh Milk	=	7400	bacteria.
Sterilisation 1 =		85	,,
,, 2 =		670	,,
,, 3 =		1500	,,

Experiment C :—

Temperature, 65° C.

Fresh Milk	=	6000	,,
Sterilisation 1 =		5	,,
,, 2 =		30	,,
,, 3 =		200	,,

These experiments are typical of the results obtained by this method. There was a marked fall in the number of organisms after the first sterilisation, but this was followed by a steady increase. At 55° C. the keeping power of the milk was not increased to any appreciable extent, and it was already sour before the second sterilisation, nor were any reliable results obtained at 60° C. When heated to 65° C., the milk, if kept in an ice chest, usually remained sweet for a week, including the days of sterilisation. In no case was an absolute sterility obtained. The results were irregular and of an inconstant nature, as the above figures illustrate (Expers. A and B). Variations occurred according to the relative resistance of the organisms that happened to be present in the milk. There was no advantage to be gained over the shorter Pasteurising process, and in practice time and expense have to be considered as well.

The milk was next subjected to a prolonged heating, at comparatively low temperatures, and a few instances of these experiments will suffice. About one pint of milk was used in each instance. The number of bacteria was estimated in a loopful of the milk. It was found that if the number of colonies appearing on the gelatine plates was counted after two or three days incubation, a quite erroneous impression was obtained as to the real number of organisms remaining alive. Thus a count made on the third day gave 70 colonies, and the same plate on the seventh day showed 140 colonies, or double the number. We therefore kept all plate cultures for a week, and the results detailed below indicate the number found on the *seventh* day.

Experiment A :—

Temperature, 55° C. for one hour.
Fresh milk = 1140 bacteria.
Sterilised ,, = 145 ,,

Experiment B :—

Temperature, 55° C. for two hours.
(1) Fresh milk = 450 bacteria.
Sterilised ,, = 98 ,,
(2) Fresh milk = 3000 ,,
Sterilised ,, = 293 ,,

Experiment C:—

Temperature, 55° C. for three hours.

(1) Fresh milk = 800 bacteria.
Sterilised ,, = 80 ,,
(2) Fresh milk = 1040 ,,
Sterilised ,, = 125 ,,

A temperature of 60° C. for one hour was also tried and the results did not materially differ from those obtained at the lowest temperature. There always remained the same residue of resistent forms (about 10 per cent.), that eventually produced in each instance a curdling of the milk, slow liquefaction of the casein and a cheesy smell. Samples of the milk so treated could be preserved for seven to fourteen days, if they were kept very cool. Such a method has naturally no practical advantage over Pasteurisation, besides being more expensive and tending to alter the flavour of the milk. As a matter of fact, we found that temperatures ranging from 60° to 68° C. applied for twenty to thirty minutes, produced equally good results, inasmuch as about 90 per cent. of the organisms were weeded out of the milk, leaving, as already stated, a residue of resistent forms or their spores. It is advisable to fix the Pasteurising temperature at 68° C., in order to make certain of killing any pathogenic organisms that may happen to be present.

Dr. Russell[1] gives the following statement of the average effect he found Pasteurisation to have on bacterial life in milk:—

	No. of Bacteria per cubc. in Unpasteurised.	Pasteurised.	Reduction in per cent.
Milk ..	3,674,000 ..	6,140 ..	99·832.

The milk was heated to 68° C. for twenty minutes.

We now proceed to describe the method of sterilisation finally adopted by us. The aim was to obtain, if possible, a simple and practicable method of heating the milk at or about 70° C. for a short period. It was hoped that a better sterilisation might thereby be obtained, without any physical alteration in the milk. In the apparatus described by Bitter the milk was treated in bulk and mechanically stirred to ensure a uniform heating. The heating was effected by

[1] *Centralblatt f. Bakteriologie*, 2te Abtheil. Bd. i., p. 741.

means of steam passing through a metal coil. It occurred to us to try the effect of reversing this process, *i.e.*, to pass the milk itself through the coil instead of the steam. A thin stream of milk passing through such a coil, heated on its outer surface, would probably become quickly warmed, and if by this means the desired temperature could be obtained with a sufficiently rapid flow, a cooked taste might at the same time be avoided. It was first necessary to determine what temperatures could be obtained by this means, and in how far it was possible to regulate the amount of heat, as well as the effect produced on the sterility of the milk. A single glass coil was made out of about six feet of glass tubing and surrounded by a water jacket. The water was heated to boiling point, and the milk was then run through the coil from a receiver. The milk was allowed to run through without applying any *vis a tergo*, and became heated to 83° to 85° C. The number of organisms in a small loopful was 200, and after sterilisation only one colony appeared on the gelatine plates, which was kept at 22° C. for seven days. The milk, however, was overheated, and had a scalded taste. The experiment was nevertheless encouraging, inasmuch as the short heating had so markedly lessened the number of bacteria. The effect of various temperatures in the coil was then investigated. It was also found necessary to apply some *vis a tergo* to the milk, and this was conveniently done by means of an air bellows worked by the foot. The results are given in the following table, the plate cultures being counted on the seventh day:—

EXPERIMENTS WITH ONE GLASS COIL.

Milk.

Temperature.	Unsterilised.	Sterilised.
75° C.	(1) 700 bacteria	12 bacteria.
	(2) 600 ,,	30 ,,
71° C.	(1) 500 ,,	35 ,,
	(2) 800 ,,	34 ,,
68°-70° C.	(1) 200 ,,	22 ,,
	(2) 200 ,,	14 ,,
68° C.	(1) 1,000 ,,	279 ,,
	(2) 2,000 ,,	297 ,,
	(3) 600 ,,	50 ,,
67° C.	(1) 1,000 ,,	85 ,,
	(2) 900 ,,	66 ,,
65°-66° C.	(1) 451 ,,	50 ,,
	(2) 544 ,,	60 ,,

The milk samples at 68° to 70° C. remained unaltered in appearance and taste, and though exposed in a beaker at room temperature were quite sweet on the following day, whilst the untreated samples were curdled. The method appeared capable of yielding good results, and we proceeded to work out the details more fully. The data obtained will be found in the following epitome :—

The effect of the initial temperature of the milk upon the temperature attained in the coil was tested. The coil was submerged in boiling water, and the milk passed through the apparatus in about ten seconds.

Initial temperature of milk = 13° C. after 68-69° C.
,, ,, = 14° C. ,, 70° C.
,, ,, = 16° C. ,, 69-70° C.
,, ,, = 26° C. ,, 72-73° C.

It will be seen that by raising the initial temperature of the milk a higher temperature is obtained in the coil, and almost any heat can be obtained by simply regulating the length of the coil, *i.e.*, by making it longer or shorter. No difficulty was found in obtaining the Pasteurising temperature, and 70°-72° C. can be safely used without altering the quality of the milk. The short heating not having affected the milk in any way, suggested the possibility of giving two short heatings instead of one by means of a double coil.

The cooling of the milk is an important point to be attended to and this was obtained by passing it through a coil submerged in water or ice after each heating—whereby the milk was subjected to an alternate heating and cooling. The apparatus thus took the following form :—

(1) A heating coil.
(2) A cooling coil.
(3) A heating coil.
(4) A cooling coil.

Thermometers were inserted at convenient points to observe the temperature.

Experiment A :—

Temperature 83-84° C.
Fresh milk (1) = 800 bacteria.

Fresh milk (2) = 1,000 bacteria.
Sterilised milk (1) = 1 ,,
,, ,, (1) = 0 ,,

Experiment B :—

Temperature 82-84° C.
Fresh milk (1) = 500 ,,
,, (2) = 600 ,,
Sterilised milk (1) = 0 ,,
,, ,, (2) = 0 ,,

The time of flow through the apparatus was 36-37 seconds. The above temperatures were too high for our purpose, but the complete absence of growth on the gelatine plates after such a short exposure of the milk to the heat was a striking result. The temperature was lowered and modified as desired by making the hot coil shorter and the cold coil longer.

A few experiments were made with two heating and three cooling coils, and the temperature noted viz. :—

(1) Hot coil 74° C.
(2) Cold ,, 18° C.
(2) Hot ,, 71° C.
(3) Cold ,, 24° C.

Result:—

Fresh milk (1) = 1,087 bacteria.
,, (2) = 880 ,,
Sterilised milk (1) = 4 ,,
,, ,, (2) = 6 ,,

The time of flow through this apparatus was 1 minute.

The following results will further illustrate the possibilities of the method—

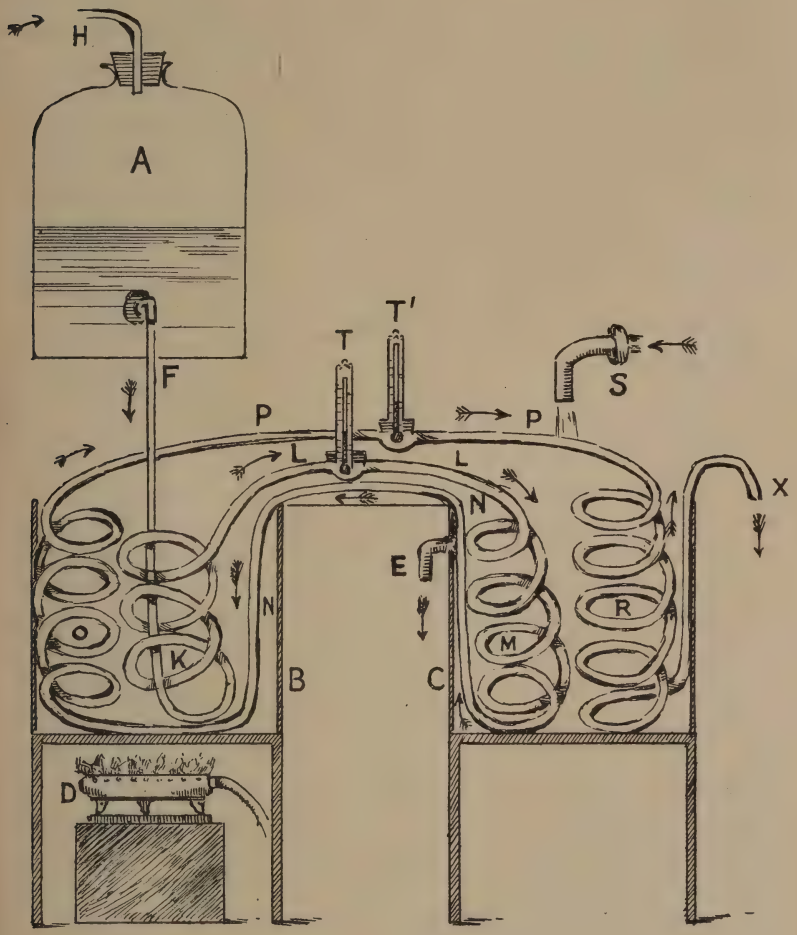
EXPERIMENTS WITH 2 HOT AND 2 COLD COILS.

Milk.

Temperature.	Unsterilised.	Sterilised.
72°-74° C.	(1) 350 bacteria	4
	(2) 370 ,,	3
68°-70° C.	(1) 370[1] ,,	5
	(2) 477[1] ,,	20
64°-68° C.	(1) 700 ,,	80
	(2) 897 ,,	25
	(3) 800 ,,	27
67°-68° C.	(1) 552[1] ,,	4
	(2) 375[1] ,,	8

[1] Rapid liquefaction of the gelatine plate.

A sample of milk was heated in the apparatus to 70° C., when the number of bacteria fell from 185,000 to 340 in a cubc. *i.e.*, a reduction of about 98 per cent.



The results were equal to those obtained by the ordinary methods of Pasteurisation, the percentage reduction in the number of bacteria being always as great and frequently better. The exposure to heat was shorter, the method

quicker and unlimited quantities of milk could be dealt with. A glass apparatus, however, is fragile, and we accordingly made experiments with metal tubing. Lead ("compo") was used as it is readily moulded to the required shape and the coils can be easily lengthened or shortened as may be found necessary. The apparatus eventually adopted consisted of two heating and two cooling lead coils. The illustration gives its component parts (*v.* p. 93). The apparatus consists of a reservoir for the milk, A, and of two water baths, B and C, containing coils of "compo" tubing of 7 mm. diameter. The water bath B is filled with water, which is kept boiling by the gas stove D, while the water bath C contains cold water which is continuously renewed from the cold water supply S, the excess running away by the pipe E. If desired the water can be further cooled with ice. The tubing forming the coils and connections is a continuous piece attached to the bottom of the milk reservoir A, passing to both water baths and ending at the exit X, so that any fluid in A has to pass through the coils in both water baths before escaping at the exit X. The method of using the apparatus is as follows. The water bath B being filled with water, which is kept boiling by the gas stove, and the water bath C *being empty*, some boiling water, about one litre, is passed through the tubing. This flushes and sterilizes the whole of the tubing. When the flushing water has passed through, the water bath C is filled with cold water, which is continuously renewed from the supply S, and flows away by E. The end of the tubing F is now attached to the bottom of the reservoir A containing the milk. The milk is forced through the tubing by connecting the top of A with an ordinary blow-pipe bellows by a tube H, and then working the bellows. The milk passes from A down F and through the coil K in the hot water bath, then through L to the coil M in the cold water bath, back again by N to the second coil O in the hot water bath, returning by P to the second coil R in the cold water bath and thence out by the exit X, where it can be caught in any desired manner. The thermometers T and T^1 are inserted into the tubes L and P coming from the hot coils K and

O, so that the temperature of the milk after heating is ascertained. By adjusting the rate of flow of the milk, the length of the coils and the size of the tubing, any desired temperature can be attained. In the cold water bath these factors are so adjusted that the milk is cooled down to the temperature of the cooling water. The following results were obtained at different temperatures with this apparatus. The average time of passage through the whole apparatus was thirty seconds. The temperature may vary a little during the operation, but this does not matter so long as it keeps within the right limits for a successful Pasteurisation, viz., 68-70 or 72° C.

Temperature. Coil 1.	Coil 2.	Unsterilised.	Sterilised.
77° ..	90° C. ..	820 bacteria	0
73° ..	90° C. ..	200,000 ,, ...	860
77° ..	87° C. ..	700,000 ,, ...	3,340
73° ..	86° C. ..	360,000 ,, ...	2,620
75° ..	78° C. ..	1,000,000 ,, ...	6,350
73° ..	76° C. ..	? ,, ...	26
71° ..	74° C. ..	60,000 ,, ...	5,000
71° ..	73° C. ..	267,000 ,, ...	1,370
70° ..	73° C. ..	88,000 ,, ...	172
68° ..	73° C. ..	3,230 ,, ...	110
68° ..	71° C. ..	300,000 ,, ...	2,800

The colonies on the plates were counted on the seventh day. We found on making comparative experiments that the results were just as good as those obtained by Pasteurisation of the milk for twenty to thirty minutes, at 68° C., *e.g.* :—

Fresh Milk.	Pasteurised.	Coil Method.
80,800	.. 161	170 bacteria
300,000	.. 3,700	2,800 ,,

The temperature we would recommend is 70° C. This temperature effects all that Pasteurisation is capable of accomplishing in the short period of thirty seconds, and does not alter the appearance or flavour of the milk. Higher temperatures do not present any practical advantage, nor do they materially alter the results. In practice the temperature may vary from 68° to 72° C., but with no injurious effect on the milk. An occasional bad result occurred in the course of the experiments, but this was due to an inferior sample of milk or the presence of a large number of resistent organisms.

A careful attention to cleanliness in milking, &c., would tend to ensure uniformity, and the sterilisation would yield still better results than we have mentioned if carried out at the dairy farm.

The milk kept for at least a week, if care was taken to keep it cool, and, as a general rule, for a longer time than that Pasteurised in the ordinary way. It may be that the combined effect of the sudden heating and cooling, twice repeated, has a more injurious influence on the spores and resistent forms present than the continuous application of heat.

The method at any rate satisfies all the ordinary commercial requirements of Pasteurisation. It remained to determine in how far it met the hygienic requirements, and for this purpose experiments were made with several pathogenic organisms as detailed below.

(1) *Diphtheria.*

(*a*) 100 cc. of a forty-eight hours' broth culture of virulent diphtheria bacilli (0·25 cc. kills guinea-pigs in twenty-six hours) were added to 1,200 cc. sterilised milk, and the mixture passed through the apparatus.

Temperature.—Coil I., 75-74° C.
,, ,, II., 70-69° C.

Two control guinea-pigs inoculated with the mixture *before passage*, receiving respectively 2·5 and 5 cc., died in forty-eight hours. Three serum tubes inoculated with the mixture *before passage*, gave a confluent growth of diphtheria bacilli. Four guinea-pigs inoculated with the mixture *after passage*, receiving respectively 5 cc., 5 cc., 6 cc. and 6 cc., were alive and well three weeks after. Four serum tubes inoculated each with several loops of the mixture *after passage*, did not show a single colony of diphtheria bacilli.

(*b*) Similar to *a*. Similar amounts of diphtheria broth, &c., and virulent bacilli. Two control guinea-pigs inoculated with 2 cc. and 4 cc. of the mixture, died in forty-eight hours. Three guinea-pigs inoculated with 5 cc., 5 cc.

and 6 cc. of mixture *after passage*, were alive and well a month after. Three serum tubes inoculated from the mixture *before*, gave a confluent growth of diphtheria bacilli, and a similar number *after passage* gave no colonies.

Temperature.—Coil I., 70° C.
,, ,, II., 70° C.

(2) *Typhoid.*

Six hundred cc. sterilised milk were mixed with 60 cc. of a thirty-hours broth culture of typhoid bacilli. Three agar plates were made from mixture *before passage*, viz. :—

Plate I.—3 loops.
,, II.—3 loops from I.
,, III.—4 loops from II.

These agar plates, incubated at 37° C., and examined after twenty-four hours, gave—

Plate I. Confluent growth.
,, II. 900 colonies.
,, III. 300 colonies.

After passage four agar plates were made :—

Plate I. 1 loop
,, II. 2 loops
,, III. 4 ,,
,, IV. 6 ,,
} = not a single colony developed in either.

Temperature.—Coil I. 70° C.
,, ,, II. 70° C.

(3) *Tubercle.*

A richly tubercular sputum was dried over sulphuric acid finely powdered and mixed thoroughly with sterilised milk. Two guinea-pigs, inoculated with 2 cc. (each) *before* passage, died exactly a month after. The glands, spleen, mesentery liver, lungs, and peritoneum were riddled with tubercle. Three guinea-pigs, inoculated each with 2 cc. *after passage*, showed no trace of tubercle after a careful *post-mortem*.

Temperature.—Coil I. 70° C.
,, ,, II. 70° C.

(4) *Staphylococcus pyogenes aureus.*

This organism was also destroyed by a similar treatment. The method, therefore, fulfils the hygienic requirements as well as the commercial, an exposure to 70° C. for thirty seconds being sufficient to completely kill the above pathogenic organisms. The sudden alternate heating and cooling of the milk during its passage proved quickly destructive. Bitter, as the result of experiments with his apparatus, found that at least twenty minutes were necessary to kill tubercle bacilli with certainty.

The apparatus we have devised is of simple construction, and is easily worked, its action is rapid, and unlimited quantities of milk can be dealt with. The commercial and hygienic requirements of Pasteurisation are met, and, as described, the machine can be automatically cleaned and disinfected before use. The temperature to be employed is 70° C. Any variations in results that occur are due to the varying bacterial quality of the milk. The difficulties that may arise from time to time depend not so much upon the number of bacteria present as upon their nature, *e.g.*, production of spores, &c.

These hindrances to a successful sterilisation, can be greatly lessened by care in milking and cleanliness in the dairy.

The experiments detailed were carried out in the laboratory, and should the opportunity present itself of conducting them on a large scale the method can then be put to a final test.

ON THE STERILISATION OF WATER BY FILTRATION.

BY JOSEPH LUNT, B.SC., F.I.C., F.C.S.

THE following pages record the results of an independent investigation of the capabilities of the Berkefeld Filter, made in the Water Laboratory of this Institute, in order to obtain an independent confirmation of results obtained by Dr. Hans Schoefer, of Vienna, and others, as to the power of this filter to intercept pathogenic bacteria—especially typhoid—even when water bacteria are able to pass through.

The experiments made may be divided under the following heads:—

(1) Does the Berkefeld Filter when freshly sterilised give a filtrate which is *absolutely* sterile?

(2) Provided the filtrate is absolutely sterile on commencing to use a freshly sterilised filter, for how long does the filter continue to yield such a sterile filtrate?

(3) Does the Berkefeld Filter show any differential action on pathogenic organisms as against the numerous harmless water bacteria which are normally present in drinking-water?

(4) Are the various methods at present employed in using the Berkefeld Filter equally efficacious, if not, which is the best method?

(5) Can any practical recommendations be made as to the best mode of using, and as to the requisite frequency of cleaning and sterilising the filter in order that it shall be an efficient safeguard against water-borne disease?

As regards the first point, *Does the Berkefeld Filter when freshly sterilised give a filtrate which is absolutely sterile?*

It may not be out of place to describe the experimental methods employed in giving an answer to this question.

The first point of importance is to secure that the filtered water shall be received and conveyed in perfectly sterile vessels, screened in an efficient manner from all chances of aerial contamination. The author has found that germs falling into water from the air undergo extensive multiplication, and where a filtrate is under examination for some days or weeks, it is essential that this source of error should be guarded against, and that all aerial contamination of filtrates be rigorously excluded. In ordinary domestic use the filtered water is delivered into a contaminated atmosphere, and such filtrates would soon contain bacteria in considerable numbers, even though none had passed through the filter. Mere sterilisation of the delivery end of the outlet tube is insufficient, as the contamination soon affects the whole bulk of filtered water. The pipettes used in measuring the several volumes of the filtrate and in transferring them to culture media must also be efficiently sterilised and similarly protected against aerial contamination. The pipettes used were plugged with wool at the upper end, and several of them were enclosed in an outer glass tube also plugged with cotton wool. The tube full of pipettes was then sterilised in the hot air steriliser, and the pipettes taken out with the proper precautions as required. The pipettes usually held 6 cc., and were convenient for sowing three culture tubes with 1, 2 and 3 cc. of filtrate respectively, from one pipette full of filtrate.

Fig. 1 shows one form of apparatus employed in the case of water artificially infected with various pure cultures of bacteria, or in the case of broth cultures.

The flask C contains the unfiltered water or broth containing bacteria: it holds 3,000 cc., and is furnished with a syphon and an air-pressure tube passing to the bottom of the liquid. When the lower end of the air tube is at the same level as the liquid which just covers the filter F, the filtration proceeds automatically, when once started by blowing the liquid over, and the filter is always covered with liquid, which flows out of C just at the same rate as the water is filtered.

Fig. 1.

There is thus an automatic delivery of unfiltered water or broth to replace that filtered no matter how the speed of filtration may vary.

This method was found particularly useful in the filtration of turbid broth in which case the rate of filtration is constantly diminishing owing to the collection of bacterial slime on the outside of the filter.

The flask F, with the filtering candle attached and furnished with a cotton-wool plug P for the filtration of air, is first sterilised by steaming for an hour on three successive days. The air which enters the flask on cooling after sterilisation, is rendered sterile by filtration through plug P. The sterilisation of C is of no consequence, as it contains the unfiltered fluid.

The filtration is carried out by partially exhausting F by means of a water pump; atmospheric pressure then forces the unfiltered water through the filter.

In these experiments the effective pressure on the filter never exceeded three-quarters of an atmosphere, and usually was well below half an atmosphere.

The filtered liquid was abstracted from F as required for examination by removing plug P with aseptic precautions and inserting a sterilised pipette for the withdrawal of the liquid, after which the plug was quickly replaced.

The most rigorous test to which a filter can be subjected is to filter a recent broth culture of an organism and to keep the filtered broth in an incubator of suitable temperature. Then if a single living bacillus be present in the litre or more of filtered broth, a turbidity will appear in twenty-four or forty-eight hours. If such a turbidity is not observed, then the filtered broth is absolutely sterile.

Many trials of the Berkefeld Filter were made in this manner with broth cultures,[1] and it was found that when properly carried out every bacillus was arrested and a sterile filtrate obtained.

Broth cultures of B. fluorescens liquefaciens,
B. mesentericus vulgatus,
B. Subtilis,
B. lactis cyanogenus.

[1] For the purpose of separating bacterial enzymes from the bacteria.

were completely sterilised by simple filtration in this way, as also were suspensions in water of:

B. coli communis,
B. typhi abdominalis,
B. prodigiosus,
B. violaceus,
B. lactis cyanogenus,
B. fluorescens liquefaciens,
Spirillum Choleræ Asiaticæ,
B. fluorescens non-liquefaciens.

500 cc. of a five days' old broth culture of B. fluorescens liquefaciens was filtered through a No. 13a candle. The broth came through perfectly clear and limpid, and remained clear in the incubator.

After twenty-four hours 3·1 cc. of the filtrate was distributed in varying volumes into seven sterile melted gelatine tubes. These remained clear and sterile, but became liquefied without turbidity owing to the presence of the soluble bacterial enzyme present in the filtered broth.

The filter which contained the bacterial mud on the outside of it was placed in the incubator for twenty-four hours at 23° C.; then a litre of London tap-water was filtered through the candle and the bacterial mud on the outside of it and 1·8 cc. of the aqueous filtrate transferred to four plates. They all became liquefied owing to the presence of the enzyme produced by the bacilli, and which had passed as a soluble chemical product through the filter; but no turbidity or fluorescent appearance manifested itself as would have been the case if the filtrate had contained any living bacilli.

This experiment was repeated by the filtration of 1000 cc. of a five days' old broth culture of B. fluorescens liquefaciens through a No. 13*a* filter. The filtrate remained absolutely clear in the incubator for two days.

The filter with the bacterial mud on the outside of it was placed in the incubator for two days. After this period 500 cc. of sterile broth was passed through the filter and the bacterial mud coating it.

From this second filtrate five plates were made containing 0·2, 0·5, 0·5, 1 cc. and 1 cc. of filtrate respectively.

All the plates became liquefied by the soluble enzymes produced by the bacilli, but the last three only became turbid and fluorescent. The first two plates remained clear, sterile, and free from fluorescence although liquefied by enzyme action. This shows that although the filtrate under these conditions had not remained absolutely sterile, yet it contained only one, or at most two bacilli per cubic centimetre. The 500 cc. of filtered broth in the above experiment became turbid in twenty-four hours at 23° C. and plates from this yielded a pure culture of the organism which had been used in the experiment.

These experiments show that when the pores of the filter are filled with nutrient broth and the outside of the filter strongly charged with bacilli, none pass through in twenty-four hours, but a few do so in two days.

The author has frequently used various forms of the Berkefeld filtering candle for the filtration of broth cultivations of various organisms, so as to separate the chemical products from the bacteria themselves, and finds that the filtrate comes through perfectly sterile, and may be kept for an indefinite length of time without any of the bacilli making their appearance in the liquid, provided proper precaution is used in conducting the filtration and avoiding all chances of contamination of the filtrate by foreign organisms. It is essential that none of the unfiltered broth shall come in contact with the india-rubber joint connecting the filtering candle with the sterile receiving vessel, as certain failure is the result, sufficient liquid being drawn in by capillary attraction to contaminate the filtrate.

A litre of London tap-water was artificially impregnated with the surface growth of three agar-agar tubes, six days' old, containing pure cultures of bacillus fluorescens liquefaciens (a common water bacillus). The resulting liquid was turbid and contained countless myriads of the bacilli. The number of bacilli present per cubic centimetre was enormously greater than would be the case with the foulest sewage. This liquid was filtered through one of the No. 13*a* filters which had previously been sterilised, and the resulting filtrate was examined for the presence of the organism which

was to be found in such countless numbers on the outside of the filter.

A volume of 11·5 cc. of the filtrate was distributed in varying proportions into twelve tubes of melted sterile nutrient gelatine, and these were poured into so many sterile Petri's dishes. These were then placed in the incubator for five days. After this period *not a single colony of the bacillus was present, and thus it was proved that the filter had removed the bacilli from the water completely.* A similar test was made with bacillus fluorescens non-liquefaciens with a like result.

Another experiment was made in a similar manner with London tap-water artificially impregnated with bacillus coli communis, but not a single bacillus could be detected in 12 cc. of the filtrate, although $\frac{1}{20}$ cc. of the water outside the filter was shown by plate culture to contain the bacilli in countless numbers.

In addition to the above experiments as already stated, suspensions in water of B. coli communis, B. typhi abdominalis, B. fluorescens liquefaciens, B. prodigiosus, B. lactis cyanogenus, Spirillum choleræ asiaticæ, B. violaceus, all from young cultures, were filtered, and in each case a perfectly sterile filtrate was obtained with the freshly sterilised filters. In these cases 12 cc. and sometimes 18 cc. was usually taken for sowings.

Of all the forms of the Berkefeld filter there is one in particular which is likely to be put to the most severe test, and on the proper performance of which there should not be the slightest mistrust. I refer to that form which is called the "Travellers' Filter" designated in the catalogue "N." This is the form which would most probably be chosen by travellers going to foreign countries, in which not only is the ordinary risk of infection by water much greater than here in England, but there is the added risk which comes from a frequent scarcity of water and its frequent most obvious pollution.

It was of importance therefore to make a thorough examination of this filter on which so much may depend, and the following experiments were made with it.

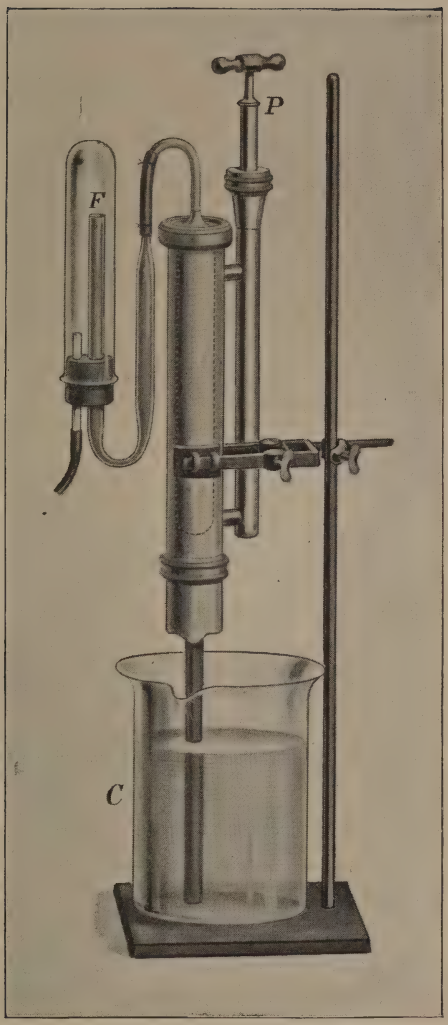


Fig. 2.

Sterilisation.—In each case before using a filter for the purposes of these experiments care was taken to thoroughly cleanse the filter from any soluble matter which may have been used in its manufacture, and which may have exerted an antiseptic action, by passing a current of clean tap-water through the filter both inwards and outwards. After a thorough cleansing, the filters were fitted with glass tubes in such a manner that the filtrate could be delivered into a germ free atmosphere, and be efficiently protected from any ærial contamination during the time they were under examination (see fig. 2).

The accompanying figure shows how the outlet tube for filtered water was protected by an outer covering tube. The outer tube was only removed for the short time required to take out a portion of the filtrate from F by means of a sterilised pipette, after which the outer tube was again replaced. The water rises in the tube as in a spring, and it is almost impossible for a contamination to occur during the abstraction of a portion of filtrate, as any dust falling on the surface of the water would at once be washed away.

The filtering candle with its glass tubes as shown in fig. 2, was sterilised by heating in an autoclave under a pressure of two atmospheres of steam corresponding to a moist heat of 130° to 134° C. for an hour. It should be noted that these filters are supplied for use with a combined suction and force pump; the intention being to make the filter available where no water pressure is to be had, *e.g.*, where the only source of water is a pool, lake, or running stream. A little consideration will show that this method of using the filtering candle is one which places the filter under some disadvantage.

All who are familiar with filtration of any kind, will recognise that for efficient filtration the process should be conducted as steadily as possible and no violent disturbance of the liquid under filtration should be allowed.

In this suction and force-pump method it will be recognised that the filtration is intermittent, and that at one moment there is a very great pressure placed on the outside of the candle, whilst in the next it is succeeded by a period of back pressure which lasts until the ball valves are again closed.

There are *à priori* reasons then for anticipating failure when such a method is adopted in using the filter. The violent and sudden changes in pressure would be expected to act prejudicially on the efficiency of filtration.

Such in fact was the result, for it was proved conclusively that bacteria were able to pass through the filter *from the very commencement of filtration*, and it was proved that this result was not owing to any inherent defect in the filtering candles themselves, because they were afterwards *proved to give a sterile filtrate* when freshly sterilised, when another method of filtration was adopted in which these violent changes of pressure did not occur.[1]

Rate of Filtration.—In this form of filter the rate is dependent solely on the number of strokes of the pump per minute, and on the efficiency of the valves. It is independent of the closeness of the pores of the filter.

With the pump used the delivery was found to be 35 to 36 cc. per stroke. 2,000 cc. was delivered in 100 seconds with 56 strokes of the pump.

The delivery of filtered water was 1,000 to 1,500 cc. per minute with ordinary working (according to rate of strokes).

In order to ascertain whether any difference existed in the closeness of the filtering material of the three candles tested, they were examined under a constant water pressure equivalent to 430 mm. of mercury.

The pressure was applied *inside* the filter and the water allowed to flow outwards and fall into a 2,000 cc. measuring flask.

No. 1 yielded 2,000 cc. in 5·33 min. (mean of 3).
„ 2 „ „ „ 4·5 „ „
„ 3 „ „ „ 6·8 „ „

The results are in accordance with these rates, for whilst Nos. 1 and 2 passed B. violaceus and B. prodigiosus respectively, No. 3 failed to pass B. lactis cyanogenus when first used by the suction and force pump method, although it

[1] On this defective method of working being pointed out to the Berkefeld Company by the author, they at once took steps to remedy the defect. The sale of the old form is now discontinued, and a new form with an air chamber fitted to it is now supplied. This tends to equalise the pressure on the filter.

allowed some water bacteria to pass. The following are the details of the experiments :—

FIRST SERIES. FILTER "N," No. 1.

Suction and Force-pump method. Bacillus Violaceus.

(Showing the passage of B. violaceus through the filter from the commencement of filtration when used by the suction and force pump method.)

100 cc. of a nine days' old broth culture was added to 1,900 cc. of unsterilised London tap-water. From the *Unfiltered water* three plate cultures were made. The first one only contained 0·1 cc. of the unfiltered water, the second a single platinum loopful, the third just the trace of liquid taken up on a thin straight sterile platinum wire. After incubation, even the third plate was crowded with colonies of bacillus violaceus as a pure culture showing that the unfiltered fluid contained countless myriads of the bacilli.

Filtered Water.

The 2,000 cc. of infected water was first filtered, and the last portion of filtrate examined in 6 cultures, viz., 3 plate cultures of 1 cc., 2 cc., 3 cc.; 3 broth cultures of 1 cc., 2 cc., 3 cc. After these cultures had been made from the filtrate a second time[1] (as above), another 6,000 cc. of unsterilised tap water was passed through the filter, in order to wash out all traces of the 100 cc. of broth used in infecting the water with the culture.

After washing in this manner, the filter with the bacilli on the outside was allowed to remain standing in unsterilised tap-water at the room temperature for two days, after which period the filtrate was tested as before, 12 cc. of filtrate being used in six cultures.

First Filtrate. First day of using Filter.

All the broths were turbid on the second day, and B. violaceus was demonstrated in cultures made from them.

[1] A second 2,000 cc. of tap water having been filtered.

The three gelatine plates showed an average of fourteen to seventeen colonies of B. violaceus per cc.

Note.—This filtrate showed, in addition to B. violaceus, the same small yellow colonies of the water bacillus noticed subsequently in Filter No. 3. They amounted to over twenty colonies per cubic centimetre, making a total of about thirty-five colonies per cc. Only these two species were found in the filtrate.

Second Filtrate. First day of using Filter.

Same results as above with the three broth cultures. The three gelatine plates showed twenty to twenty-two colonies of B. violaceus per cc.

Note.—This filtrate also showed the small yellow colonies mentioned above.

Third Filtrate. Two days after first using Filter.

Broths 1 cc., 2 cc., 3 cc. All turbid after three days' incubation at 23° C. Control cultures, as before, showed B. violaceus.

Gelatine plates 1 cc., 2 cc., 3 cc. All crowded with the characteristic colonies of B. violaceus after fourteen days' incubation, showing a large increase in the number of bacteria in the filtrate in two days.

SECOND SERIES. FILTER "N," No. 1.

Suction Method. Bacillus Fluorescens Liquefaciens.

(Same filtering candle as in Series I., B. violaceus.)

[Showing the production of a filtrate which is sterile on the first day of using the filter, practically sterile[1] on the first and third days after first using, and non-sterile on the fourth day. In these experiments the suction and force pump method was discarded, and atmospheric pressure used to force the water through the filter into a partially exhausted receiver.]

[1] Only one or two organisms in 12 cc. of filtrate.

Several loops of a two days' old culture of bacillus fluorescens liquefaciens on agar were suspended in 1,000 cc. of unsterilised London tap-water. A thin, straight sterile platinum wire dipped in the unfiltered water, then stirred into melted gelatine and poured on a sterile plate gave a culture crowded with the characteristic colonies of the organism used.

Filtered Water.

First Filtrate. First day of first using Filter.

Effective Pressure 300 mm. of Mercury.	Duration of Incubation at 23° C.				
	24 Hours.	3 Days.	4 Days.	6 Days.	7 Days.
Broths 1 cc.	Clear ..	Clear ..	Turbid[1]		
,, 2 cc.	,, ..	,, ..	Clear ..	Clear ..	Clear.
,, 3 cc.	,, ..	,, ..	,, ..	,, ..	,,
Gelatine Plates 1 cc.	No Colonies ..		No Colonies ..		No Colonies.
,, ,, 2 cc.	,, ,, ..		,, ,, ..		,, ,,
,, ,, 3 cc.	,, ,, ..		,, ,, ..		,, ,,

Second Filtrate. First day after first using Filter.

Effective Pressure 300 mm. of Mercury.	Duration of Incubation at 23° C.		
	2 Days.	3 Days.	5 Days.
Broth 1 cc.	Clear	Clear ..	Clear.
,, 2 cc.	Turbid at Surface[2]		
,, 3 cc.	Clear	Clear ..	Clear.
Gelatine Plates 1 cc. ..	No Colonies ..	No Colonies..	No Colonies.
,, ,, 2 cc. ..	,, ..	,,	,,
,, ,, 3 cc. ..	,, ..	,,	,,

[1] Plates made from this broth showed absence of B. fluorescens liquefaciens, and as the broth only became turbid on the fourth day, and as the 2 cc. and 3 cc. broths remained clear, this result is most probably due to an accidental contamination; the colonies had no liquefying power, and the plate cultures confirmed this.

[2] Plate cultivations made from this broth showed a pure cultivation of bacillus fluorescens liquefaciens and the turbidity was in all probability caused by a single bacillus in the 2 cc. of filtrate used for infecting broth No. 2. If there had been 2 bacilli in the 6 cc., the 3 cc. broth would have become turbid in all probability, therefore the 12 cc. of filtrate only contained one bacillus.

Third Filtrate. Third day after first using Filter.

Effective Pressure 249 mm. of Mercury.	Duration of Incubation at 23° C.			
	24 Hours.	3 Days.	4 Days.	10 Days.
Broth, 1 cc.	Clear ..	Turbid[1]		
,, 2 cc.	,, ..	Clear ..	Clear ..	Clear.
,, 3 cc.	,, ..	,, ..	,, ..	,,
Gelatine Plates 1 cc.. .	No Colonies	No Colonies	No Colonies	No Colonies.
,, ,, 2 cc...	,,	,,	,,	,,
,, ,, 3 cc...	No Colonies visible	1 Colony of B. fluorescens liquefaciens.		

Therefore, these filtrates, although not absolutely sterile, are exceedingly poor in bacteria, only containing one or two bacilli in 12 cc. of filtered water.

Fourth Filtrate. Fourth day after first using Filter.

Duration of Incubation at 23° C., 2 Days.

Broth, 1 cc. } All turbid and fluorescent { Plate cultures showed pure cultures of B. fluorescens liquefaciens.
,, 2 cc. }
,, 3 cc. }

Gelatine Plates, 1 cc. } Crowded with B. fluorescens liquefaciens the 3 cc. plate was completely liquefied, the 2 cc. plate almost all liquefied, and the 1 cc. crowded with colonies almost coalescing.
,, ,, 2 cc. }
,, ,, 3 cc. }

THIRD SERIES. FILTER "N," No. 2.

Suction and force pump method. Bacillus Prodigiosus.

[Showing the passage of B. prodigiosus through the filter from the commencement of filtration when used by the suction and force pump method.]

100 cc. of a ten days' old broth culture was made up to 2,000 cc. with unsterilised London tap-water, and from the unfiltered water a trace was taken on a sterile straight platinum wire and sown in a plate culture. In twenty-four hours the plate was pitted all over with the colonies of B. prodigiosus.

Two other control plates made with a loop-ful and 0·1

[1] The above broth proved to be a pure culture of B. fluorescens liquefaciens. This filtrate therefore only showed two bacilli in 12 cc. of water.

cc. respectively were completely liquefied in twenty-four hours, showing that the unfiltered water swarmed with B. prodigiosus.

Filtered Water.

First Filtrate. First day of using Filter.

Duration of Incubation at 23° C., 24 Hours.

Broths, 1 cc.	Turbid	A loop-ful of turbid liquid streaked on agar showed presence of B. prodigiosus after Incubation.		
,, 2 cc.				
,, 3 cc.				
Gelatine Plates, 1 cc.—2 days.			20 liquefied colonies of B. prodigiosus	Control cultures on agar and potato show B. prodigiosus.
,, ,, 2 cc.		,,	30 liquefied colonies of B. prodigiosus	
,, ,, 3 cc.		,,	Completely liquefied	

Second Filtrate. First day of using Filter.

Duration of Incubation at 23° C., 24 Hours.

Broth, 1 cc.	Turbid	A loop-ful of turbid broth streaked on agar from each ..	After 4 days the agar cultures show the characteristic red growth of B. prodigiosus.	
,, 2 cc.				
,, 3 cc.				
Gelatine Plates, 1 cc.—2 days.			Very crowded, and pink tinge visible	Control agars show B. prodigiosus.
,, ,, 2 cc.		,,	Completely liquefied and pink tinge visible	
,, ,, 3 cc.		,,		

Third Filtrate. Twenty-four hours after first using Filter.

Duration of Incubation at 23° C.

	3 Days.	4 Days.
Broth, 0·5 cc.	Turbid	All turbid, fluorescent at surface and film tinged pink.
,, 1 cc.		
,, 2 cc.		
Gelatine Plates, 0·5 cc.	All plates completely liquefied, very fluorescent, and showing tinge of red on surface	Both B. prodigiosus and B. fluorescens liquefaciens present both in broth and plate cultures.
,, ,, 1 cc.		
,, ,, 2 cc.		

In the above series the filtration was conducted exactly as in the first series (see ante), and the results show that B. prodigiosus passed through the filter from the very commencement of filtration, and also that this organism was accompanied by B. fluorescens liquefaciens from the unsterilised water twenty-four hours after first being used.

FOURTH SERIES. FILTER "N," No. 2.[1]

Suction Method. Bacillus Violaceus.

[Showing the sterility of the filtrate on first day of using, and showing the passage of bacillus violaceus through the filter two days after first being used, at the same time bacillus fluorescens liquefaciens began to appear in the filtrate in exceedingly small numbers.]

Several loop-fuls of a twenty-four hours' growth of B. violaceus on potato were suspended in 1,000 cc. of unsterilised London tap-water and the turbid water filtered. The *unfiltered water* was proved to be swarming with B. violaceus by plate culture as before. The plate cultivation also showed the presence of B. prodigiosus in the unfiltered water owing to the flask infected with B. violaceus having previously been used to contain a suspension of that organism and not having been subsequently sterilised.

Filtered Water.

First Filtrate. First day of using Filter.

Effective Pressure 256 mm. of Mercury.	Duration of Incubation at 23° C.				
	2 Days.	3 Days.	5 Days.	7 Days.	10 Days.
Broth 1 cc.	Clear ..	Clear ..	Clear ..	Clear..	Clear.
,, 2 cc.	,, ..	,, ..	,, ..	,, ..	,,
,, 3 cc.	,, ..	,, ..	,, ..	,, ..	,,
Gelatine Plates 1 cc. ..	No Colonies	No Colonies	No Colonies	No Colonies	No Colonies.
,, ,, 2 cc. ..	No Colonies	No Colonies	No Colonies	No Colonies	No Colonies.
,, ,, 3 cc. ..	No Colonies	No Colonies	No Colonies	No Colonies	No Colonies.

Second Filtrate. Second day after first using Filter.

	Duration of Incubation at 23° C.	
	24 Hours.	4 Days.
Broth, 1 cc.	Clear	Turbid. Plate culture from this broth shows pure B. violaceus.
,, 2 cc.	,,	,, Control potato cultures give the blue growth of
,, 3 cc.	,,	,, B. violaceus.
Gelatine Plates, 1 cc.	8 days	Average number of colonies of B. violaceus[2]
,, ,, 2 cc.		= 10 per cc. Plate 2 showed one colony of
,, ,, 3 cc.		B. fluorescens liquefaciens.

[1] Same filtering candle as in Third Series.

[2] Control cultures from the surface colonies were made on potato, and these gave the characteristic growth of violaceus after two days' incubation.

Third Filtrate. Three days after first using Filter.

	Duration of Incubation at 23° C.	
	3 Days.	
Broth, 1 cc.	Turbid.	Plate cultures give pure B. violaceus.
„ 2 cc.	„	Control cultures on potato give the characteristic growth of B. violaceus.
„ 3 cc.	„	
Gelatine Plates, 1 cc.	7 days.	All the plates show the presence of B. violaceus.
„ „ 2 cc.		(Potato controls used). Average number only 2 or 3 colonies per cc. No colonies of B. fluorescens liquefaciens noted.
„ „ 3 cc.		

FIFTH SERIES. FILTER "N," No. 3.

Suction and Force pump method. Bacillus Lactis Cyanogenus.

[Showing that this filter, although used by a disadvantageous method, but being a "closer" filtering candle entirely keeps back bacillus lactis cyanogenus on the first day of using, but gives a filtrate which is not absolutely sterile, owing to the presence of a small water bacillus in the filtrate. After standing three days in unsterilised water the filtrate shows the presence of B. lactis cyanogenus in small numbers, but bacillus fluorescens liquefaciens is predominant.]

100 cc. of an eight days' old broth culture of bacillus lactis cyanogenus was added to 1,900 cc. of unsterilised London tap-water and filtered. From the *unfiltered water* four plate cultures were made containing respectively 0·2 cc., 0·1 cc., a loop-ful, and a straight wire dip. Even the last one was very crowded with the characteristic colonies of B. lactis cyanogenus.

The filtration was carried out exactly as in the first series. After the first trial the filter was allowed to remain standing in unsterilised tap-water for three days before the next trial was made.

Filtered Water. First day of using Filter.

	Duration of Incubation at 23° C.			
	24 Hours.	3 Days.	4 Days.	15 Days.
Broth, 1 cc. ..	Clear..	Clear..	All very ..	Loop smears on agar show absence of B. lactis cyanogenus.
,, 2 cc. ..	,, ..	,, ..	slightly ..	
,, 3 cc. ..	,, ..	Turbid	turbid ..	
Gelatine Plates,[2] 1 cc.	No colonies			No B. lactis cyanogenus detected.
,, ,, 2 cc.	,,			
,, ,, 3 cc.	,,			

Second Filtrate. First day of using Filter.

	Duration of Incubation at 23° C.			
	24 Hours.	3 Days.	4 Days.	15 Days.
Broth, 1 cc. ..	Clear..	Clear..	Very[2] ..	Loop smears on agar show absence of B. lactis cyanogenus.
,, 2 cc. ..	,, ..	,, ..	slightly ..	
,, 3 cc. ..	,, ..	,, ..	turbid ..	
Gelatine Plates,[2] 1 cc.	No colonies			No B. lactis cyanogenus present.
,, ,, 2 cc.	,,			
,, ,, 3 cc.	,,			

The above results show that on filtering water strongly impregnated with B. lactis cyanogenus through a freshly sterilised filter, none of the bacilli could be detected in 24 cc. of the filtrate although a water bacillus was found. After the first day's trials the filter was allowed to remain in unsterilised tap-water for three days.

[1] Plates made from these broth tubes showed no B. lactis cyanogenus but only the small yellow colonies of the small water bacillus noted below.

[2] In all these plates minute slow-growing colonies appeared averaging about six colonies per cubic centimetre. The broths also gave a pure culture of the same organism on plate cultivation.

There can be little doubt that this small slow-growing bacillus was derived from the unsterilised tap-water.

Third Filtrate. Third day after first using Filter.

	Duration of Incubation at 23° C.	
	3 days.	4 days.
Broths, 1 cc.	All turbid and fluorescent	Slight film, all turbid and very fluorescent, probably almost pure, B. fluorescens liquefaciens.	No plates made from these broths, as B. fluorescens liquefaciens was so predominant.
,, 2 cc.		
,, 3 cc.		
Gelatine Plates,[1] 1 cc.	Half liquefied by B. fluorescens liquefaciens. One colony of B. lactis cyanogenus found.	
,, ,, 2 cc.	Liquefied almost completely by B. fluorescens liquefaciens.	
,, ,, 3 cc.		

It is evident, therefore, that three days after first being used this filter gave a filtrate in which B. fluorescens liquefaciens was predominant, but in which, nevertheless, B. lactis cyanogenus could with certainty be detected although it must have been present in very small numbers.

SIXTH SERIES. FILTER "N," No. 3.

Suction Method. Bacillus Prodigiosus.

[Showing the production of a sterile filtrate on the first and second days of using filter, practical sterility on the third day and great bacterial purity on the fourth day.]

Several loops of a two days' old potato culture of bacillus prodigiosus suspended in 1,000 cc. of unsterilised tap-water and filtered. From the *unfiltered water* a control plate culture was made on the first day with a single loop-ful of fluid. The plate became almost completely liquefied in twenty-four hours, and was tinged the characteristic pink due to B. prodigiosus. After three days the "well" was again examined for B. prodigiosus, and a straight wire dip gave a plate crowded with that organism in twenty-four hours.

[1] From these plates 3 small non-liquefying colonies were sown on gelatine but only one of these proved to be B. lactis cyanogenus.

Filtered Water.

First Filtrate. First day of using Filter.

Effective Pressure 255 mm. of Mercury.	Duration of Incubation at 23° C.				
	24 Hours.	3 Days.	6 Days.	7 Days.	11 Days.
Broth 1 cc.	Clear..	Clear..	Clear..	Clear..	Clear.
,, 2 cc.	,, ..	,, ..	,, ..	,, ..	,,
,, 3 cc.	,, ..	,, ..	,, ..	,, ..	,,
Gelatine Plates 1 cc. ..	No Colonies			No Colonies.	
,, ,, 2 cc. ..	,, ,, ...			,, ,,	
,, ,, 3 cc. ..	,, ,, ...			,, ,,	

Second Filtrate. First day after first using Filter.

Effective Pressure 296 mm. of Mercury.	Duration of Incubation at 23° C.				
	2 Days.	3 Days.	5 Days.	6 Days.	10 Days.
Broth 1 cc.	Clear..	Clear..	Clear..	Clear..	Clear.
,, 2 cc.	,, ..	,, ..	,, ..	,, ..	,,
,, 3 cc.	,, ..	,, ..	,, ..	,, ..	,,
Gelatine Plates 1 cc. ..	No Colonies			No Colonies.	
,, ,, 2 cc. ..	,, ,, ...			,, ,,	
,, ,, 3 cc. ..	,, ,, ...			,, ,,	

Third Filtrate. Third day after first using Filter.

Effective Pressure 285 mm. of Mercury.	Duration of Incubation at 23° C.			
	24 Hours.	3 Days.	4 Days.	8 Days.
Broth 1 cc. ..	Clear ..	Clear.. ..	Clear ..	Clear.
,, 2 cc. ..	,, ..	,, ..	Turbid[1]	
,, 3 cc. ..	,, ..	,, ..	Clear ..	Clear.
Gelatine Plates 1 cc. ..	No Colonies			No Colonies.
,, ,, 2 cc. ..	,, ,, 1 dot Colony[2]..			
,, ,, 3 cc. ..	,, ,, ...			No Colonies.

[1] Plate culture and control on potato made, but no B. prodigiosus present.
[2] Not B. prodigiosus which rapidly liquefies gelatine.

Fourth Filtrate. Fourth day after first using Filter.

	Duration of Incubation at 23° C.		
	2 Days.	3 Days.	7 Days.
Broth 1 cc.	Clear ..	Clear ..	Clear.
,, 2 cc.	,, ..	,, ..	Turbid } Not B. prodigiosus, nor
,, 3 cc.	,, ..	,, ..	Turbid } B. fluorescens liq.
Gelatine Plates 1 cc.	No Colonies seen.		*** B. prodigiosus is absent as it reveals itself as a rapidly liquefying colony in 24 hours.
,, ,, 2 cc.			
,, ,, 3 cc.			

Fifth Filtrate. Eight days after first using Filter.

	Duration of Incubation at 23° C. 3 Days.
Broth 1 cc.	All turbid and fluorescent, no pink film seen. — Plate cultivation showed great predominance of B. fluorescens liquefaciens, no B. prodigiosus could be detected.
,, 2 cc.	
,, 3 cc.	
Gelatine Plates 1 cc.	Great predominance of B. fluorescens liquefaciens. No. B. prodigiosus detected on the rapidly liquefying plates.
,, ,, 2 cc.	
,, ,, 3 cc.	

Experiments with Bacillus Coli Communis.

Bacillus coli communis may be taken as a type of those organisms which are not normal in good potable water; it is normal in human excreta, constantly present in the alimentary canal, and has by many been taken as evidence of pollution when found in water. It thrives best at blood heat, and is allied to the bacillus of typhoid.

Bacillus coli communis is particularly suitable as an example of a foreign organism in water in experiments on the relative behaviour of such organisms and those which are normal in water when subjected to filtration, for it can be readily detected and separated from water which contains a host of other species of organisms usually found there.

Method of Separation and Recognition.

Unless natural water contains B. coli communis in large numbers ordinary plate cultivation is inadequate for its detection, as other organisms grow rapidly and soon monopolise a plate culture for themselves, and their presence necessitates the use of minute volumes of water for such plate

cultures in which the B. coli communis may perchance not be present. Special methods are, however, available by which B. coli communis can be separated from all other bacteria in water, under conditions which are favourable to its growth and multiplication, whilst inimical to the life of most other organisms usually present in water, I refer to Parietti's method. Parietti's solution contains :

Crystallised Carbolic Acid	50 grams
Hydrochloric Acid	40 grams
Water	1,000 cc.

If this solution be added to ordinary nutrient broth in the proportion of 0·3 cc. to 10 cc. of broth we have a suitable medium for the rapid growth at 37° C. of B. coli communis, and if a natural water or one artificially sown with this organism be added to such a medium, most other organisms are killed, whilst a very rapid multiplication of B. coli communis takes place. Such a broth tube becomes turbid in twenty-four hours and often gives off minute bubbles of gas, especially on shaking the tube. Plate cultures made from such a turbid broth often give pure cultures of coli which, if the normal variety of this organism, may be verified by sowing in :

(1) Milk, which is curdled in twenty-four hours to forty-eight hours.

(2) Sugar litmus agar, which is turned acid, becomes riddled with gas bubbles in twenty-four hours, and remains acid.

(3) Broth and peptone water, which give the indol reaction in twenty-four hours to forty-eight hours.

(4) Melted and resolidified nutrient gelatine, riddled with gas bubbles in twenty-four hours.

(5) Gives a rapid and characteristic growth on nutrient gelatine in plates and streak tubes, &c.

This method was used in examining the unfiltered water used in the following experiments, and by its means B. coli communis was detected with certainty thirty-nine days after the water was first infected with this organism.

During this time, however, it was rapidly declining in numbers, and it ultimately dies out altogether.

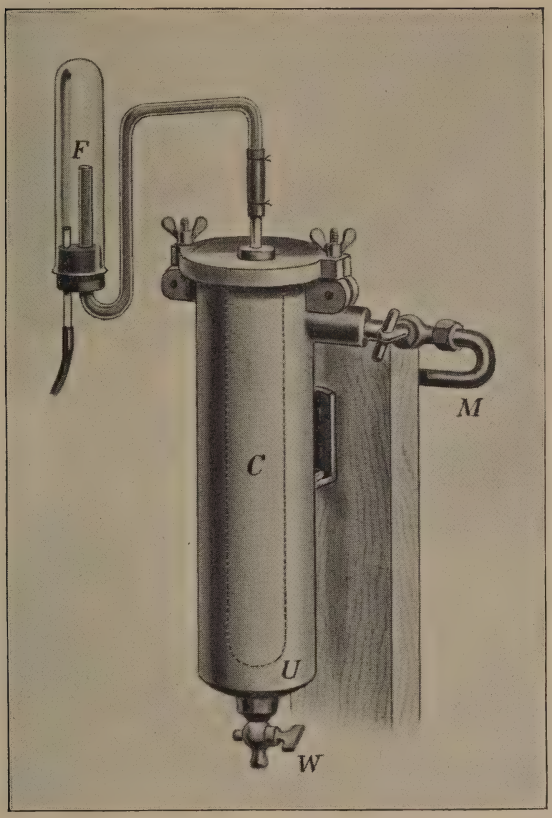


Fig. 3.

During this period (thirty-nine days) in which B. coli communis was proved to be constantly present on the outside of the filter, it was never once detected, although most carefully searched for in the filtered water. The normal inhabitants of water have the best chance of surviving in the filtered water, whilst foreign organisms are unable to survive the adverse conditions.

Method of Experiments.

Figure 3 shows the form of filter employed in these experiments; it is known as the Household Filter "H," but has a 13A candle fitted to it. The water as before is delivered into a germ free atmosphere and the whole arrangement is sterilised in an autoclave, except the outer iron casing.

Unfiltered Water.[1]

First day of using Filter. Bacillus Coli Communis.

A carbolised gelatine plate, containing 0·1 cc. of Parietti's solution to 10 cc. of nutrient gelatine sown with 0·1 cc. of the unfiltered water became very crowded with colonies which were a pure cultivation of B. coli communis.

The colonies were in two cases sown on the various media and demonstrated to be B. coli communis. A similar plate sown with 0·5 cc. of the unfiltered water became crowded with colonies of B. coli communis.

Seven days after first using Filter.

Coating of bacteria on outside of filter undisturbed.

Water outside filter drawn off by flushing tap W.

A carbolised gelatine plate (0·1 cc. of Parietti's solution to 10 cc. of nutrient gelatine sown with 0·1, 0·5 and 1 cc. of unfiltered water) gave from 50 to 60 colonies of B. coli communis per cubic centimetre.

[1] The unfiltered water was infected with several loops of the surface growth from a young sloped gelatine culture.

A carbolised broth tube (0·1 cc. of Parietti's solution to 10 cc. of nutrient broth) sown with 0·1 cc. of unfiltered water (also one 0·5 cc.) became turbid in twenty-four hours; plate cultures made from it gave a pure and characteristic cultivation of B. coli communis. The nature of the colonies was confirmed by the various tests for this organism. Carbolised broths containing 0·2 cc. of Parietti's solution to 10 cc. of broth also became turbid in twenty-four hours after infection with 1 cc. and 2 cc. of unfiltered water. Bacillus coli communis was also detected on the ninth and eleventh days.

Unfiltered Water.

Twenty-six days after first using Filter. B. Coli Communis.

I. Coating of Bacteria outside filter undisturbed. Unfiltered water drawn off from outside filter by flushing tap.

Broth.	Parietti's Solution.	Unfiltered Water.	Incubated 17 Hours.	2 Days.
10 cc.[1] ..	0·1 cc. ..	1 cc. ..	Slightly turbid	
,, [1] ..	,, ..	2 cc. ..	Distinctly turbid	
,, ..	0·2 cc. ..	1 cc. ..	Clear	Clear.
,, ..	,, ..	2 cc. ..	,, ...	,,
,, ..	0·3 cc. ..	1 cc. ..	,, ...	,,
,, ..	,, ..	2 cc. ..	,, ...	,,

Gelatine Plates.

Gelatine.	Parietti's Solution.	Unfiltered Water.	Incubated 3 Days.
10 cc. ..	0·1 cc. ..	0·5 cc. ..	No Colonies } Only an organism of
,, ..	,, ..	1 cc. ..	1 Colony } B. subtilis group.
,, ..	,, ..	2 cc. ..	3 Colonies } No coli detected.
,, ..	0·2 cc. ..	0·5 cc. ..	
,, ..	,, ..	1 cc. ..	6 days—all sterile.
,, ..	,, ..	2 cc. ..	

[1] Examined microscopically on the fourth day, and found to be a motile spore forming organism. Plate cultures from these broths and also the carbolised plates only show an organism of the B. subtilis group and no B. coli communis.

II. Drippings from outside filtering candle when filtered water is forced through reverse way.

Broth.	Parietti's Solution.	Unfiltered Water.	Incubated 17 Hours.	2 Days.
10 cc.[1] ..	0·1 cc. ..	0·5 & 1 cc...	All very turbid, and gas evolved on slightly shaking the tube.	
,, [1] ..	0·2 cc. ..	0·5 & 2 cc...		
,, ..	0·3 cc. ..	1 cc. & 2 cc.	.. ..	Clear.

Gelatine.	Parietti's Solution.	Water.	6 days.	17 days.
10 cc...	·1 cc...	1 cc...	100 Colonies of Coli, confirmed	
,, ..	·2 cc...	2 cc...	No Colonies	A dot Colony proves to be Coli.

Thirty-nine days after first using Filter.

I. Coating of Bacteria on outside of filter undisturbed. Unfiltered water drawn off from outside of filtering candle by the flushing tap.

Broth.	Parietti's Solution.	Unfiltered Water.	24 Hours.	10 Days.
10 cc.	0·1 cc. ..	1 cc. ..	Turbid.[2]	
,,	,, ..	2 cc. ..		
,,	,, ..	3 cc. ..		
,,	0·2 cc. ..	1 cc. ..	Clear ..	Clear.
,,	,, ..	2 cc. ..	Turbid ..	Contained only an organism of the B. subtilis group.
,,	,, ..	3 cc. ..		

[1] Plate cultures from these tubes showed characteristic pure cultivations of B. coli communis, which were confirmed by cultivation on other media.

[2] B. coli communis was not detected in the plate cultivations from these broths, but only an organism of the B. subtilis group, which liquefied gelatine.

II. Drippings from outside the filtering candle when filtered water is forced through reverse way.

Broth.	Parietti's Solution.	Unfiltered Water.	
10 cc.	0·1 cc.	½ cc. and 1 cc. ..	All Turbid.[1]
,, ...	,, ...	2 cc.	
,, ...	,, ...	3 cc.	
,, ...	0·2 cc.	½ cc. and 1 cc. ..	All Turbid.[1]
,, ...	,, ...	2 cc.	
,, ...	,, ...	3 cc.	

Carbolised gelatine plates containing 0·15 cc. of Parietti's solution also gave pure cultures of B. coli communis when sown with 1·5 cc. of the drippings.

Similarly a plate with 0·1 cc. of Parietti's solution and 1 cc. of water gave coli. From the above results it is clear that although B. coli communis could be detected in the unfiltered water itself seven days after first using the filter, it could not be so detected on the twenty-sixth and thirty-ninth days, but only when the bacterial coating on the outside of the filter was washed off and examined. On doing this, however, B. coli communis was found still to be present in the living state, though it was apparently in not so vigorous a condition, as it was unable to grow in a 3 per cent. Parietti's broth, which it is usually able to withstand.

FILTER 13A.

Bacillus Coli Communis.

Normal Culture Media (without Parietti's Solution).

Filtered Water.

First three days during which Filter was in use.

Broth at 23° C. { 1 cc. 2 cc. 3 cc. } Broth at 37° C. { 1 cc. 2 cc. 3 cc. } Gelatine Plates at 23° C. { 1 cc. 2 cc. 3 cc. }

For the first three days on which the filter was used all the culture tubes remained sterile. On each day 18 cc. of filtrate was used for the sowings, 6 cc. in three broth tubes

[1] Plates were made from these (when twenty-four hours old) turbid broths, and in twenty-four hours they gave pure cultivations of B. coli communis, which were verified by further confirmatory cultures on the various media.

(1, 2 and 3 cc.) at 23° C., a like amount in three broth tubes at 37°, and a further like amount in three plates, all the broths remained clear and the plates sterile.

On the fourth day after first using the filter sowings were made as before. The broth tubes at 23° C. became turbid, the 1 cc. tube also became fluorescent, and the plates each showed but one liquefying colony.[1]

Sowings were made in carbolised broth containing 1 per cent. and 2 per cent. of Parietti's solution from the broths turbid at 23°. These also remained clear, showing the absence of B. coli communis.

On the fifth and seventh days after first using the filter the plate cultures showed mainly B. fluorescens liquifaciens, but no B. coli communis was detected.

Sowings made in carbolised broth tubes as before, from the broths which became turbid at 37°, remained clear, proving the absence of B. coli communis. The broth tubes incubated at 23° C also became turbid, but were not further examined. On the seventh day after first using the filter B. coli communis was demonstrated readily in the unfiltered water without disturbing the bacterial coating on the outside of the filter.

Twenty-four carbolised broth cultures containing 0·1 cc. and 0·2 cc. of Parietti's solution in parallel sets and sown with 1, 2 and 3 cc. of filtrate remained clear after sowing with filtrate taken from the filter, 12, 15, 26 and 39 days after commencement of filtration, showing the absence of B. coli communis.

[1] The broth tubes at 37° C. remained sterile, showing that the organisms which had passed through the filter could not live at that temperature, evidently being water bacteria, which thrive best at ordinary temperatures. The inability to grow at 37° C. proves that the organisms present could not have been B. coli communis.

FILTER 13A.

Bacillus Coli Communis. Filtered Water (continued). Uniform pressure 430 mm. Outside pressure method. Carbolised Broth Tubes at 37° C.

Volume of Broth.	Volume of Parietti's Solution.	Volume of filtrate added.	No. of days after commencement of Experiment.	Duration of Incubation at 37° C.				
				2 days.	3 days.	6 days.	16 days.	25 days.
10 cc. ..	0·1 cc...	1 cc.	9 days[1] ..	Clear..	Clear	Clear	Clear	Clear[2]
,, ..	,, ..	2 cc.	,, ..	,, ..	Clear	Slightly turbid ..	Loops of fluid on Gelatine streak. Few Colonies *not* Coli ..	
,, ..	,, ..	3 cc.	,, ..	,, ..	Clear			
,, ..	0·2 cc...	1 cc., 2 cc., and 3 cc.	,, ..	—Clear for 25 days.[2]				
				1 day.	4 days.	5 days.	14 days.	23 days.
10 cc. ..	0·1 cc...	1 cc.	11 days[1] ..	Clear..	Clear..	Clear	Clear	Clear[2]
,, ..	,, ..	2 cc.	,, ..	,, ..	,, ..	,, ...	,, ...	,,
,, ..	,, ..	3 cc.	,, ..	,, ..	Turbid	Plates show one species only not Coli		
,, ..	0·2 cc...	1 cc., 2 cc., and 3 cc.	.. ..	—Clear for 23 days.[2]				

[1] B. coli communis was detected with certainty in the water which was allowed to drip from the *outside* of the filter on both these occasions; 10 cc. broths containing 0·2 cc. of Parietti's Solution became turbid in 24 hours, and yielded pure cultivations of B. coli communis on being examined by plate culture. B. coli communis was not, however, detected in the unfiltered water taken from the outside of the filter without disturbing the bacterial coating on the filter itself.

[2] Re-infected with one loop of 24 hours' broth culture of bacillus coli communis; all turbid in 17 hours.

Experiments with the Typhoid Bacillus.

Unfiltered Water. Carbolised Broth Tubes.

The "well" water was infected with several loops full of a young agar-agar culture of the typhoid bacillus 24 hours before commencing filtration.

First Day of using Filter.

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.	2 Days.
10 cc. ..	0·2 cc. ..	0·1 cc. ..	Turbid.	
,, ..	,, ..	0·2 cc. ..		
,,[1] ..	0·25 cc. ..	0·2 cc. ..		
,,[1] ..	,, ..	0·5 cc. ..		
,, ..	0·3 cc. ..	0·2 cc. ..	Clear for 22 days.	
,,[1] ..	,, ..	0·5 cc. ..	Clear	Turbid.

Second Day of using Filter. (In use One Day).

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.
10 cc. ..	0·2 cc.	0·1 cc.	Turbid.
,, ..	,, ...	0·2 cc.	
,,[1] ..	0·25 cc.	0·2 cc.	
,,[1] ..	,, ...	0.5 cc.	
,, ..	0·3 cc.	0·2 cc.	Clear for 10 days.
,,[1] ..	,, ...	0·5 cc.	Slightly turbid.

Third Day of using Filter. (In use Two Days).

Broth.	Parietti's Solution.	Well Water.[2]	2 Days.	3 Days.
10 cc. ..	0·2 cc.	0·1 cc.	Turbid ..	Turbid.
,, ..	,, ...	0·2 cc.	Clear ..	
,,[1] ..	0·25 cc.	0·2 cc.	Turbid ..	
,,[1] ..	,, ...	0·5 cc.	,, ..	
,, ..	0.3 cc.	0·2 cc.	Clear for 20 days.	
,, ..	,, ...	0·5 cc.		

Fourth Day of using Filter. (In use Four Days).

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.
10 cc. ..	0.2 cc.	0·1 cc.	Turbid.
,, ..	,, ...	0·2 cc.	
,,[1] ..	0·25 cc.	0·2 cc.	
,,[1] ..	,, ...	0·5 cc.	
,, ..	0·3 cc.	0·2 cc.	Clear for 7 days.
,,[1] ..	,, ...	0.5 cc.	Slightly turbid.

Fifth Day of using Filter. (In use Five Days).

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.
10 cc. ..	0·2 cc.	0·1 cc.	Turbid.
,, ..	,, ...	0·2 cc.	
,,[1] ..	0·25 cc.	0·2 cc.	
,,[1] ..	,, ...	0·5 cc.	
,, ..	0·3 cc.	0·2 cc.	Clear for 17 days.
,, ..	,, ...	0.5 cc.	

Sixth Day of using Filter. (In use Six Days).

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.
10 cc. ..	0.2 cc.	0·1 cc.	Turbid.
,, ..	,, ...	0·2 cc.	
,,[1] ..	0·25 cc.	0·2 cc.	
,,[1] ..	,, ...	0·5 cc.	
,, ..	0·3 cc.	0·2 cc.	Clear for 16 days.
,, ..	,, ...	0·5 cc.	

Seventh Day of using Filter. (In use Seven Days).

Broth.	Parietti's Solution.	Well Water.[2]	24 Hours.
10 cc. ..	0·2 cc.	0·1 cc. and 0·2 cc...	Turbid.
,,[3] ..	0·25 cc.	0·2 cc. and 0·5 cc...	
,, ..	0·3 cc.	0·2 cc. and 0·5 cc....	Clear for 15 days.

Eighth Day of using Filter. (In use Nine Days).

Broth.	Parietti's Solution.	Well Water.[2]	2 days.
10 cc. ..	0·2 cc.	0·1 cc.	Turbid.
,, ..	,, ...	0·2 cc.	
,,[3] ..	0·25 cc.	0·2 cc.	
,,[3] ..	,, ...	0·5 cc.	
,, ..	0·3 cc.	0·2 cc.	Clear for 13 days.
,, ..	,, ...	0·5 cc.	

Ninth Day of using Filter. (In use Thirteen Days).

Broth.	Parietti's Solution.	Well Water.[2]	2 days.
10 cc. ..	0·2 cc.	0·2 cc.	Turbid.
,, ..	,, ...	0·5 cc.	
,,[3] ..	0·25 cc.	0·5 cc.	
,,[3] ..	,, ...	1 cc.	
,, ..	0·3 cc.	0·5 cc.	Clear for 9 days.
,, ..	,, ...	1 cc.	

Tenth Day of using Filter. (In use Twenty-Six Days.)

Broth	Parietti's Solution.	Well Water.[2]	2 Days.
10 cc. ..	0·2 cc.	1 cc.	Turbid.
,,[4] ..	,, ...	2 cc.	Turbid.
,, ..	0·25 cc.	1 cc.	Clear for 14 days.
,,[4] ..	,, ...	2 cc.	Turbid.
,, ..	0·3 cc.	1 cc.	Clear for 14 days.
..	,, ...	2 cc.	

These results demonstrate that when a vigorous culture of the typhoid bacillus is introduced without food material

[1] Used for confirmation of presence of typhoid bacillus.

[2] Drippings from outside of filter, when filtered water was forced the reverse way.

[3] No Typhoid demonstrated in these broths, but only a liquefying, spore-forming organism of the B. subtilis group.

[4] In these broths the typhoid bacillus was not detected; on plate cultivation they yielded only organisms of the B. subtilis group. 1 cc. of the drippings from the outside of the filter contained no organisms capable of withstanding 0·25 of Parietti's solution.

into unsterilised water inhabited by water bacteria, the typhoid organisms first of all show a lessened vitality as is shown by their failure to withstand so large a dose of Parietti's solution as at first. Then they decline greatly in numbers and ultimately die out altogether.

Although this well-water was artificially infected with the typhoid bacillus in numbers very largely exceeding those of the water bacteria, yet the water bacteria became predominant and in less than a week the typhoid bacillus could no longer be demonstrated in the unfiltered water nor yet in the drippings falling directly from the outside of the filter.

During this time the typhoid bacillus was never once detected in the filtered water although the experiments show that the method used was adequate to its detection.

It is then but a question of "environment" and the "survival of the fittest."

That the presence of water bacteria has an adverse influence on the life of the typhoid bacillus is shown by the fact that this organism will live for a much longer time even in distilled water when the other competing forms are absent, *i.e.*, when the distilled water is sterilised before the introduction of the typhoid bacilli. In my own experiments I have been able to demonstrate the typhoid bacillus in distilled water even after seventy-six days. This proves that it is not so much the want of food material as the struggle for existence which goes on between them and the water bacteria.

It should be remembered that the typhoid bacilli in these experiments were not accompanied by B. coli communis as would be the case in a natural typhoid infection, this fact rendered the task of demonstrating the typhoid bacilli much simpler than would have been the case in a naturally infected water, as the presence of B. coli communis materially diminishes the chances of isolating the bacillus of typhoid.

The following table gives results of experiments with filtered water.

FILTER "N."

Filtered Water.

Suction Method. Bacillus Typhi Abdominalis.

First Filtrate. First day of using Filter.

	Duration of Incubation.					
Broth Incubated at 23° C.	24 Hours.	2 Days.	4 Days.	6 Days.	16 Days.	22 Days.
1 cc.	Clear ..	Clear ..	Clear ..	Clear.		
2 cc.	,, ..	,, ..	,, ..	,, ..	Clear ..	Clear.
3 cc.	,, ..	,, ..	,, ..	,, ..	,,	,,
Broth Incubated at 37° C.						
1 cc.	Turbid	Plate Cultures, not Typhoid, probably accidental contamination.				
2 cc.	Clear ..	Clear ..	Clear ..	Clear ..	Clear.	
3 cc.	,, ..	,, ..	,, ..	,, ..	,,	

Carbolised Broth Tubes at 37° C. ·2 P. to 10 cc.

1 cc. .. .. ..
2 cc. .. .. .. } All clear for 22 Days.
3 cc. .. .. ..

Gelatine Plates.	24 Hours.	2 Days.	4 Days.	6 Days.	7 Days.	9 Days.
1 cc. ..	No Colonies	No Colonies	No Colonies	No Colonies	No Colonies	No Colonies
2 cc. ..	,,	,,	,,	,,	,,	,,
3 cc. ..	,,	,,	,,	,,	,,	,,

Second Filtrate. First day after first using Filter.

	Duration of Incubation.			
Broth Incubated at 23° C.	24 Hours.	3 Days.	5 Days.	15 Day
1 cc.	Clear ..	Clear ..	Clear ..	Clear.
2 cc.	,, ..	,, ..	,, ..	,,
3 cc.	,, ..	,, ..	,, ..	,,

Broth Incubated at 37° C.	24 Hours.	3 Days.	5 Days.	15 Days.	21 Days.
1 cc.	Turbid	Plates, not Typhoid.			
2 cc.	Clear..	Turbid			
3 cc.	,, ..	Clear..	Clear..	Clear..	Clear.

Gelatine Plates, 1 cc., 2 cc. and 3 cc. gave no Colonies in three Days.
No Typhoid detected subsequently.

Third Filtrate. Second day after first using Filter.

Broth Incubated at 23° C. — 2 Days.

1 cc. .. .. .. }
2 cc. .. .. .. } Turbid near surface, fluorescent and with slight film, characteristic of B. fluorescens liquefaciens. Plate cultivations show this organism apparently in the pure state.
3 cc. .. .. .. }

Broth Incubated at 37° C.	2 Days.	4 Days.	
1 cc.	Clear	All Turbid.	Plate cultures, show no Typhoid.
2 cc.	Turbid		
3 cc.	,,		

Carbolised Broth Tubes [·2 P. to 10 cc.]	2 Days.	4 Days.	9 Days.	20 Days.
1 cc.	Clear ..	Clear ..	Clear ..	Clear.
2 cc.	,, ..	,, ..	,, ..	,,
3 cc.	,, ..	,, ..	,, ..	,,

Gelatine Plates. — Incubated 2 Days.

1 cc. .. .. Very crowded with B. fluorescens liquefaciens (pure).
2 cc. .. .. Almost completely liquefied by fluorescens liquefaciens (pure).
3 cc. .. .. Completely liquefied and fluorescent.

Fourth Filtrate. Third Day after first using Filter.

Broth at 23°	2 Days.
1 cc. ..	Turbid and fluorescent at top, slight film = B. fluorescens liquefaciens.
2 cc. ..	
3 cc. ..	

Broth at 37°	2 Days.	
1 cc. ..	All turbid	Plate cultures do not show the Typhoid bacillus, but mainly a liquefying organism.
2 cc. ..		
3 cc. ..		

Carbolised Broth at 37° C. [·2 P. to 10 cc.]	2 Days.	7 Days.	18 Days.
1 cc.	Clear	Clear	Clear.
2 cc.	,, ...	,, ...	,,
3 cc.	,, ...	,, ...	,,

Gelatine Plates.	24 Hours.	2 Days.
1 cc.	All crowded with Colonies ..	Completely liquefied and fluorescent = B. fluorescens liquefaciens.
2 cc.		
3 cc.		

Fifth Filtrate. Fourth day after first using Filter.

Broth at 23° C., *twenty-four hours*, 1 cc., 2 cc. and 3 cc., as before, B. fluorescens liquefaciens.

Broth at 37° C., 1 cc., 2 cc. and 3 cc, all turbid in *twenty-four hours.*

Carbolised broth at 37° C. [0·2 P. to 10 cc.] 1 cc., 2 cc. and 3 cc. All clear for *seventeen days.*

Gelatine plates, twenty-four hours, 1 cc., 2 cc. and 3 cc., as before, completely liquefied with B. fluorescens liquefaciens.

Further examined after being six days, seven days, nine days, thirteen days, and twenty-six days in use.

The carbolised broths containing 0·2 cc. of Parietti's solution invariably remained sterile when sown with 1 cc., 2 cc. and 3 cc. of filtrate, showing absence of the typhoid bacillus.

Experiments with the Cholera Bacillus.

Any experiments with the cholera bacillus in unsterilised water must, of necessity, be not so satisfactory as those with bacillus coli communis or the bacillus of typhoid. For the best method of detecting the cholera bacillus which is at our command, although it may answer very well for its detection in choleraic dejecta in which the bacillus is present in large numbers, cannot be relied upon as a sure and certain method for detecting cholera bacilli which are present in small numbers in unsterilised water containing a host of water bacteria. The matter is also further complicated by the fact that many cholera-like organisms have been, and may be, detected in water, which closely resemble the cholera bacillus from choleraic dejecta. All who have ex-

perimented on the behaviour of the cholera bacillus in unsterilised water agree that its life under those conditions is a short one, and that it soon dies out in the struggle with the normal bacterial inhabitants of water. Some investigators find from two to seven days to be the limit of life of the cholera bacillus in unsterilised water. So it is evident that unless the choleraic contamination is continuous, any water once infected with cholera will not retain its virulence for any lengthened period. The culture used in these experiments was one which had been passed through many artificial cultures, and so had lost some of its initial activity. It liquefied gelatine very slowly, but grew well in peptone water at 37° C., and gave a good cholera-red reaction in twenty-four hours and a good surface film of commas. Twelve platinum wire loop-fuls of such a twenty-four hours' surface film were introduced into 100 cc. of unsterilised London tap-water, and this was then made up to a litre and passed through a "N" filter by the suction method, then being followed by two litres more of unsterilised tap-water.

The filtrate was then examined as follows :—

First Filtrate. First day of using Filter.

	Period of Incubation.			
Broths at 23° C.	2 Days.	4 Days.	6 Days.	9 Days.
1 cc.	Clear	Clear	Clear	Clear
2 cc.				
3 cc.				
Broths at 37° C.				
1 cc.	Clear	Clear	Clear	Clear
2 cc.				
3 cc.				
Peptone Water at 37° C.				
1 cc.	Clear	Clear	Clear	Clear
2 cc.				
3 cc.				
Gelatine Plates.				
2 cc.	No Colonies	No Colonies	No Colonies	No Colonies
3 cc.				

Second Filtrate. Two days after first using Filter.

	Period of Incubation.			
Broth at 23° C.	24 Hours.	3 Days.	5 Days.	8 Days.
1 cc.	Clear	Clear	Clear	Clear.
2 cc.	Clear.	Became slightly turbid, with a small motile bacillus morphologically distinct from cholera, no cholera-like film.		
3 cc.				
Broth at 37° C.				
1 cc.	Clear	Clear	Clear	Clear.
2 cc.				
3 cc.				
Peptone Water at 37° C.				
1 cc.	Slowly became very slightly turbid, organisms present morphologically distinct from cholera, *no* cholera-red reaction.			
2 cc.				
3 cc.				

Gelatine Plates at 23° C.	7 Days.
1 cc.	No Colonies.
2 cc.	3 dot Colonies, not cholera.
3 cc.	4 dot Colonies, not cholera.

Unfiltered Water (Cholera Experiments).

On the second day after first using the filter the drippings from the outside of it were examined by the peptone water method at 37° C for the presence of the cholera bacillus.

After twenty-four hours the surface pellicle was examined microscopically, and it was found that the larger proportion of the organisms present were totally distinct from cholera, although a few isolated commas were seen. These were so greatly outnumbered by other bacilli that the task of separating them by plate culture was hopeless.

Fresh peptone water tubes were sown with the twenty-four hours' surface film from the first peptone waters without any better result. The organisms which had grown so enormously in the first tubes were also in great preponderance in the second series. Plate cultivations were made without the cholera bacillus being detected. It should be stated that owing to the difficulty in obtaining recent cultures of the cholera bacillus in this country direct from choleraic dejecta, it was not found possible to employ a culture which was so vigorous and healthy as it might be; and it might be worth while to repeat the experiments with the cholera bacillus when an opportunity presents itself of using really vigorous and normal cultures of the bacillus.

CONCLUSIONS AND PRACTICAL RECOMMENDATIONS.

(1) A large number of experiments have shown that when the filters are efficiently sterilised and the filtered water protected from all sources of contamination, they give on the first day of using an absolutely sterile filtrate when used in an approved manner.

This is the case with all the forms of filtering candles examined, not only with natural water but also with water artificially infected with enormous numbers of bacteria from cultures, and also with broth cultures teeming with bacilli.

In each case not a single bacillus could be detected in the filtered water from the freshly sterilised filters when proper precautions were taken in using them.

(2) Although the filter gives an absolutely sterile filtrate when used as above stated, it is necessary if such a sterile filtrate is desired continuously to re-sterilise the filter daily; for bacteria begins to appear in the filtrate in very small numbers on the second or third day of using. These bacilli are mainly, if not entirely, water bacteria.

For all domestic purposes, however, the experiments show that unless there are special circumstances which make such a precaution necessary, a thorough cleansing and sterilisation need only be performed twice or even once a week.

(3) A long series of experiments, in which London tap-water was strongly infected with bacillus coli communis, showed that this organism could not be detected in the filtered water over a period of thirty-nine days.

The method used in searching for this organism in the filtered water never failed to detect it in the unfiltered water or the bacterial coating on the outside of the filter. During this period bacillus coli communis was so detected on the outside of the filtering candle on the thirty-ninth day.

In these experiments water-bacteria could, however, be detected in the filtered water on the fifth day of using the filter, although they were present in exceedingly small numbers.

(4) Exhaustive experiments showed that with London

tap-water strongly infected artificially with the typhoid bacillus not a single typhoid bacillus was detected in the filtered water over a period of twenty-six days, though detected in the unfiltered water up to the sixth day.

As is well known typhoid bacilli ultimately perish in water which contains the water bacteria which are always present in natural water, but during the whole time in which they were demonstrated to be alive in the unfiltered water, they were never once detected in the water which had passed through the filter.

(5) London tap-water strongly infected artificially with the cholera bacillus was found after filtration through the filter to be absolutely sterile and therefore entirely free from the cholera organism.

(6) A long series of experiments with the travellers "N" filter have shown conclusively that the method of using these filters with a combined suction and force-pump, causing as it does sudden and violent changes of pressure on the outside of the filter, is one which gives very bad results with filtering candles, which give an absolutely sterile filtrate when used by a better method.

The principle of the "R" filters intended for travellers, in which atmospheric pressure forces the filtered water into an exhausted receiver is one which is free from the above objection, and when used by this method absolutely sterile filtrates are obtained even when identically the same filtering candles are used which previously passed large numbers of bacteria from the very first, when used by the "N" or suction and force-pump method.

(7) Seeing that the bacteria which first appear in the filtered water are harmless water bacteria, and that such organisms as B. coli communis and the typhoid bacillus do not accompany them, even when they are present in large numbers in the unfiltered water, it seems that in all cases in which a drinking water is not under the suspicion of being contaminated, a semi-weekly or weekly cleansing and sterilisation of the filtering candle is all that is necessary as a precautionary measure against water borne disease.

Nevertheless, in all cases of contaminated water supplies

or supplies known to be infected with disease organisms, a daily sterilisation of the filtering candle is advisable, thus ensuring an absolutely sterile filtrate.

As an additional precautionary measure in cases of epidemic outbreaks all possibility of harm from any chance defect in the working of a filter should be guarded against by boiling even the filtered water when used for drinking purposes.

Wherever a daily sterilisation of the filtering candle is resorted to, it is advisable to have two candles, one of which can be cleaned and sterilised whilst the other is in use, in order that a clean and sterile filtering candle shall be available for use immediately a used one is removed.

The volume of water required should be procured slowly at a minimum pressure and stored in a suitable manner, by which means the filter works more efficiently, rather than by the use of high pressures and a more rapid flow for a shorter period.

THE BACILLUS OF BUBONIC PLAGUE BACILLUS PESTIS.

BY RICHARD T. HEWLETT, M.D., M.R.C.P.

A CASE which occurred in London last autumn presented at the time considerable difficulty in diagnosis, and it was thought advisable that portions of the tissues should be examined for the bacillus of bubonic plague. The bacillus of bubonic plague was first described by Kitasato early in 1894. He observed it in the blood of living patients, and in the blood, buboes, and organs of those who had died of the disease. In his preliminary communication[1] (which has not, so far as I know, been followed by a subsequent one) he describes the plague bacillus as a rod with rounded ends, staining readily and showing marked polar staining, but slightly motile, and sometimes apparently capsulated. It grew freely on blood serum and agar at 37° C. as a greyish moist film, and without liquefying the former. In beef broth it makes the medium somewhat cloudy. In cultivations long chains of bacilli occur, the individual bacilli of which are so short as to be almost coccoid. Owing to the high temperature prevailing at Hong Kong, the characters of the growth on gelatin could not be ascertained. On potato there was a slight growth as a greyish film at 37°; spores were not observed. It was pathogenic for mice, rats, guinea-pigs and rabbits. Later in the same year (1894) Yersin[2] was commissioned by the French Government to investigate the outbreak of bubonic plague at Hong Kong. He described the bacillus met with in the buboes as short and thick with rounded ends, staining readily but not by Gram's method,

[1] *Lancet*, 1894, ii., p. 428.

[2] *Ann. de l'Inst. Pasteur*, viii., 1894, p. 662.

showing polar-staining, and sometimes apparently encapsuled. On gelatin it forms whitish transparent colonies, some of the colonies developing much more rapidly than others, and containing bacilli which are less virulent than those in the slower-forming colonies. In broth the growth is very characteristic, the fluid remains clear while a grumous deposit forms on the sides and bottom of the tube consisting of chains of very short bacilli, among which swollen involution forms occur. It kills mice in one to three days, rats and guinea-pigs in two to five days, rats also dying when fed with plague material; it is also pathogenic for rabbits, and in large doses for pigeons. Under cultivation the bacillus readily loses its virulence. Aoyama[1] also gives an account of the bacillus of bubonic plague based on the descriptions of Kitasato and Yersin. He considers that the bacilli met with in the blood by Kitasato, and stated by this observer to stain by Gram's method, are not identical with those present in the glands. The bacilli did not liquefy blood-serum. Yersin, Calmette, and Borrel,[2] in some work on immunisation, found that by passage through a series of animals they could obtain a "fixed virus" which killed mice in two days, young guinea-pigs in two to three days, and rabbits in three days. They were unable to obtain toxic substances from the cultivations, and for preparing an antitoxin were obliged to make use of attenuated cultures.

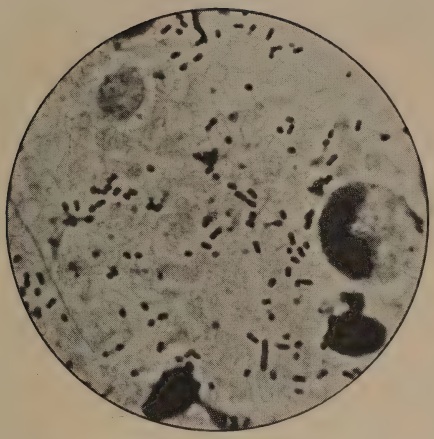
Zettnow[3] states that the bacillus of plague is non-motile, and although a thick capsule is apparently present, he was unable to demonstrate one. After twenty-one days in a gelatin stab, a snow-white growth is present.

The material from the present case, which was under the care of Dr. Patrick Manson, at the Seamen's Hospital, Albert Dock, was received in two sterilised test tubes, one containing a piece of the enlarged femoral gland, the other a small piece of the neighbouring femoral vein. An emulsion was made in sterile water with the piece of gland, and from it plate-cultivations and cultivations on various

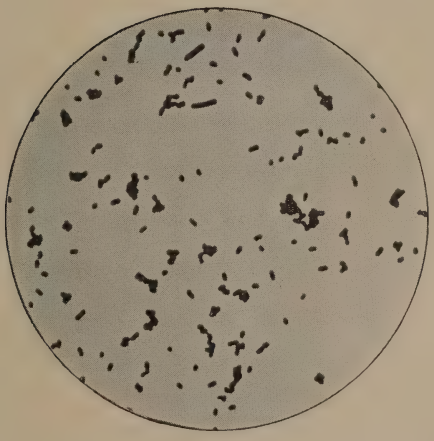
[1] *Mittheil. aus der Med. Facult. der Kaiserl. Japan. Universitat.*, Bd. iii., No. ii., p. 115.

[2] *Ann. de l'Inst. Pasteur*, ix., 1895, p. 589.

[3] *Zeitschr. f. Hygiene.*, xxi., 1896, p. 165.



Bacillus pestis x 1000. (Bubonic plague.) Cover-glass preparation from spleen of guinea-pig.



Bacillus pestis x 1000. (Bubonic plague.) Two day old agar surface culture.

media were prepared. A guinea-pig was inoculated with the remainder of this emulsion, and the piece of vein was inserted under the skin of another guinea-pig. The cultivations proved to be much contaminated, partly perhaps because, as pointed out by Aoyama, other organisms occur in the buboes, and partly because the material was not manipulated until a lapse of three or four days after death. For the latter reason no direct microscopical examination of the tissues was made. Before any likely organism had been isolated from the cultures, the guinea-pig inoculated with the gland died, so their further examination was abandoned. The animal did not show any symptoms for two or three days, it then began to be sluggish, and to have a slight discharge from the eyes, and on one occasion, on the fifth day, suffered from a curious attack—it fell on its side, the heart's action became feeble, the respiration infrequent, shallow, and gasping, and there was every appearance of a speedy ending. Recovery followed, however, and death did not take place until the seventh day, two days later.

The *post-mortem* appearances were: Hæmorrhages and great œdema at the seat of inoculation, and great enlargement of the inguinal lymphatic glands. The most striking feature was the appearance of the spleen, it was much enlarged, four to six times larger than normal, deeply congested, and studded all over with yellowish points like miliary tubercles. Microscopical examination of the fluid from the seat of inoculation, of the organs and blood, showed the presence in enormous numbers of a short thick ovoid bacillus with rounded ends, generally in pairs, so short as to appear at first sight to be a large diplococcus. Cultivations were readily obtained on various media, and the following is the detailed description of the organism :—

Morphology.—A markedly pleomorphic organism. *In the animal body* it occurs for the most part as a short, almost ovoid rod, generally linked in pairs, measuring 2·3 μ $\times$ 1·7 μ, but longer forms are to be seen here and there measuring as much as 5 μ. (See plate.)

In cultivation.—The bacilli in young cultures (twenty-four to forty-eight hours) are so short as to be almost

coccoid or slightly ovoid, on agar of about the same size as in the animal body, on gelatin, however, slightly smaller, but a few well marked rods and even threads are always to be seen sparsely distributed in the preparations. In older cultures rod, thread, and involution forms occur more numerously. (See plate.)

In broth the organism forms chains of slightly ovoid bacilli, appearing at first sight like a streptococcus. (See plate.)

Motility.—The organism is non-motile.

Capsulation. — Sometimes in drop-cultivations the organism is apparently encapsuled, but I have failed to demonstrate a capsule by staining methods.

Blood-serum cultures.—On blood-serum it forms moist, smooth, shining, cream-coloured colonies or growth, slightly raised above the surrounding medium. The blood-serum is not liquefied.

Agar Cultures.—Colonies are raised, round and cream-coloured, finely granular, denser at the centre than at the margins. Margins regular. Size 0·25—0·5 mm. in two days at 37° C.

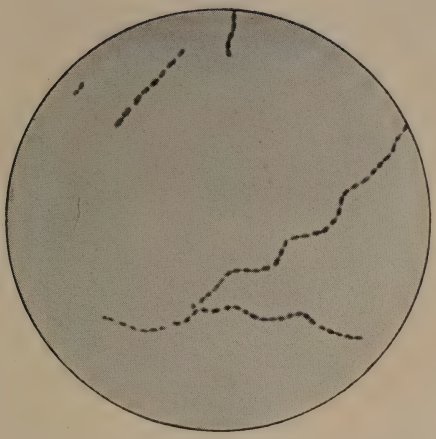
Agar Cultures (*Surface*).—Forms a thick, opaque, moist, smooth, cream-coloured growth, the margins of which are usually markedly crenated. The growth is very sticky and adherent.

Sugar Agar Cultures (*Stab*).—Grows to the bottom of the stab as a cream-coloured streak which does not diffuse into the surrounding medium. There is no development of gas.

Gelatin Cultures (*Colonies*). — Whitish, filmy, finely granular with regular margins. Size 0·1—0·25 mm. in five days at 22° C.

Gelatin Cultures (*Surface*). — Forms a thick white shining growth, with slightly irregular surface and margins, nearly confined to the inoculation track. The growth does not penetrate into the medium nor does it render it cloudy. (See plate.) The growth is very adherent.

Gelatin Cultures (*Stab*).—Delicate whitish finely granular growth, developing to the end of the stab, and with little tendency to spread from the needle-track. The gelatin is



Bacillus pestis. Three day old broth culture.
Carbol-fuchsin × 1000.

Bacillus pestis. Ten day old gelatin surface culture.

not liquefied. Both in agar and gelatin cultures, fresh punctate growths sometimes develop after a time in the original growth, simulating contaminations.

Broth Cultures.—Growth somewhat characteristic. For two or three days the broth remains perfectly clear, but a flocculent growth forms and gradually increases in amount on the bottom and sometimes on the sides of the tube. After some days the broth may become a little cloudy. No or very slight film formation.

Potato.—No growth occurs on ordinary potato.

Milk.—Milk is not coagulated.

Indol Production.—With sulphuric acid alone, a feeble indol reaction can be obtained with week-old broth cultures. With sulphuric acid and a nitrite, a well marked indol reaction can be obtained under the same conditions.

Acid Production.—With *neutral litmis agar* no acid or alkaline reaction. With *neutral litmus sugar agar* a well marked acid reaction after two days' growth.

Reducing action on Methylene Blue.—Broth cultures reduce a weak solution of Methylene Blue.

Vitality of Cultures.—Cultures retain their vitality for at least a month, but the limit has not been determined.

Pathogenesis.—Guinea-pigs die in three to seven days; if in three days the enlargement and nodulation of the spleen may not be marked. Mice die in two to three days, the spleen being much enlarged. Rats die in about a week after being fed with the organs of a guinea-pig which has died of the disease. Occasionally a very small dose may fail to kill a guinea-pig.

Distribution in the Tissues.—The organism is met with in all parts of the body. At the seat of inoculation, in the glands and spleen it is extremely abundant, less so in the liver, lung, and kidney, not nearly so numerous in the blood. The chief changes observed in the tissues are cloudy swelling and hyperæmia, and numbers of minute hæmorrhages. The organism isolated was undoubtedly the bacillus of bubonic plague, and the opportunity afforded for studying the bacteriology of the disease in this country has fortunately remained unique.

BACTERIA AND DUST IN AIR.

BY A. MACFADYEN, M.D., AND JOSEPH LUNT, B.SC.

A GREAT deal of time and attention have been devoted to the examination of the air for the presence of micro-organisms. The fact that organic matter when simply exposed to the air undergoes a fermentative or putrefactive decomposition, led to the researches, so far-reaching in their results, of Pasteur, Tyndall and Lister. The authentic proof was furnished that all such processes are due to the action of living cells, since these changes do not occur if they are excluded. The living agents at work are ubiquitous and the resulting re-distribution of matter is a universal process. The individual organisms can be isolated from the air and their functions experimentally demonstrated in the laboratory. The germs floating in the atmosphere, though invisible under ordinary conditions, are obtained as visible growths on exposing, for example, nutrient gelatine to the air, when crops of moulds, yeasts and bacteria appear. When the causal relation of bacteria to numerous diseases was proved beyond doubt, the air was naturally suspected to be one of the main channels of infection. From the earliest times great weight has been laid upon the danger of infection through air-borne contagia, and with the introduction of antiseptic surgery the endeavour was made to lessen this danger as much as possible by means of the carbolic spray, &c. In the same connection numerous bacteriological examinations of air have been made with the view of arriving at results of hygienic value. Miquel,[1] for example, has conducted a most painstaking

[1] *Reports of the Montsouris Observatory.*

series of observations regarding the bacteria present in air, their number and fluctuation according to meteorological and seasonal conditions. The air of the park at Montsouris was examined during a period of six years. The average number of bacteria found was 455 per cubic metre. The lowest mean number of organisms—155 per cubic metre—occurred in February, and the highest — 740 per cubic metre—occurred in July. The air in the centre of Paris was examined during a similar period, and the average number of organisms present was 3,910 per cubic metre. The lowest mean number — 1,800 per cubic metre—was found in January, and the highest—5,750 per cubic metre —in May. Flügge estimates the average number to be 500-1,000 per cubic metre, of which 100-200 are bacteria. If one takes the average number of bacteria present in the air as 100 per cubic metre, "a man during a lifetime of 70 years inspires about 25 million bacteria, the same number contained in a quarter litre of fresh milk, or in 25 cc. of the ordinary milk sold."[1] The number of organisms in the air is, therefore, relatively small, and of these the majority are moulds and harmless forms. The investigations made have not proved ordinary air to be one of the important channels of infection. The bactericidal action of sunlight, desiccation and the diluting action of the atmosphere on noxious substances are factors that will always greatly lessen the risk of direct infection. The majority of the organisms pass into the air with the dust from *dry* surfaces, and this in itself is inimical to a number of pathogenic bacteria. The spores of moulds and certain saprophytic bacteria are better able to withstand the absence of moisture, but the number of such organisms present in a sample of air can hardly be taken as a measure of the danger of air infection. Further, the majority of the bacteria present in inspired air are kept back at the entrance to the respiratory tract, the nose being a most efficient filter for this purpose. The researches of Drs. St. Clair Thomson and Hewlett[2] show that the nasal cavity is self-cleansing,

[1] Flügge "Grundriss der Hygiene," 1897.

[2] "Micro-organisms in the Healthy Nose," *Trans. Medical and Chirurg. Socy.*, 1895.

and that the mucous lining of the nose is practically automatically aseptic. The expired air is almost completely deprived of germs.

The majority of the bacteria present in the air are attached to the particles of dust which are continually being detached from surfaces and wafted into the atmosphere. The number of bacteria therefore increases with the amount of dust present. Air currents and the artificial agitation of the air in a dwelling increase the number of dust particles, and at the same time the number of bacteria. If, on the other hand, the air is rendered dust-free, it is deprived of practically all the micro-organisms it may contain.

In this respect rain is one of the great purifiers of the atmosphere by washing out the dust, and as regards enclosed spaces, the stilling of dust and the disinfection of surfaces liable to breed dust or to contain bacteria are more important points than air disinfection. This fact has now been recognised in modern surgery. The estimation of the amount of dust present in a sample of air would be a more reliable indicator of danger than any other test we are at present able to apply. The great majority of these dust particles are, however, invisible to the naked eye, and the estimation of their amount and the proportion of dust in the atmosphere was consequently a matter of great difficulty. The earlier methods consisted mainly in passing the air through water or over a film of glycerine. A microscopic examination was then made of the dust deposited.

We have to thank Dr. Aitken for devising a method of not only rendering the dust particles visible but also of counting them in samples of air. The ability to count the particles depends on the principle of making them visible by super-saturating with vapour, the air in which they are floating. "Under these conditions each dust particle becomes a centre of condensation, and what was invisible by itself becomes quite visible by its effects, as each invisible dust particle becomes a centre of condensation and grows into an easily visible drop."[1] The important physical laws

[1] Aitken, *Transactions, Royal Soc. Ed.*, vol. xxxv.

determining the formation of fog, mist, &c., need not be dwelt upon, as they are so well known. Dr. Aitken has been able to prove that such phenomena are due to the condensation of the atmospheric moisture upon the aërial dust particles, and do not occur in dust-free air. "If there was no dust in the air there would be no fogs, no clouds, no mist, and no rain." These facts led to the possibility of counting the dust particles themselves and of demonstrating the omnipresence of dust.

The apparatus employed is known in its latest form as "Aitken's Dust Counter."

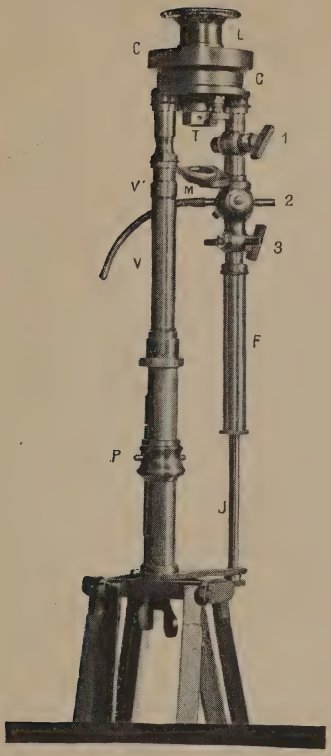
The experiments we made were primarily undertaken with a view of testing the possibility of the practical use of the instrument in hygienic investigations of the air. It would be of great value to have a ready means of testing the amount of aërial dust impurity in dwellings, factories, &c. Further, the idea occurred to us of simultaneously estimating the number of micro-organisms present in samples of air, and in this way demonstrating the ratio existing between the number of bacteria and dust particles present. The first experiments were carried out with the "Pocket Dust Counter." This apparatus is only available for counting dust particles up to 25,000 per cc., and, as London air usually contains more than this, the instrument was discarded, and the larger instrument devised by Dr. Aitken was obtained. It is known as the "Portable Dust Counter" and is available for counting dust particles up to 500,000 per cc. As will be seen from our experiments even this instrument was not always adequate for estimations in London air. The instrument is shown in the accompanying photograph. A considerable amount of practice and careful attention to sundry details in manipulation are necessary for its successful use. The first experiments made were consequently of a discouraging nature. The following description of the apparatus and the method of using it may therefore prove acceptable to others who may wish to test it.

The Portable Dust Counter (see plate) consists of a cylindrical metal chamber C, about 5·4 cubic metres in

diameter, closed at the top by a circular plate of thick glass. The bottom plate of the chamber is of metal and has a circular hole into which can be tightly screwed, a flanged brass tube T, bearing at its upper end a small circular glass plate or micrometer with its surface etched into square millimetres. The etched surface of the glass is one cubic metre below the lower surface of the large upper glass plate, when the tube carrying the former is screwed home. The upper glass plate is surmounted by a brass cap C, carrying a pair of bi-convex lenses by means of which the lines etched on the lower glass plate can be distinctly seen in focus.

The chamber capacity corresponding to a square cubic metre ruled on the lower glass plate is exactly one cc., and a square cubic metre of surface receives the falling dew drops from 1 cc. of air. One square, a square millimetre in area, receives the dew drops from $\frac{1}{100}$ cc. of the air in the chamber. The inside of the chamber is coated with bibulous paper which is moistened with water when the instrument is in use, so that the air of the chamber is saturated with water vapour. The lower metal plate of the chamber has also two openings in it, one at each side of the micrometer. The one has a small air pump screwed into it, the other possesses three taps, 1, 2, and 3, and a brass tube F, tightly packed with cotton wool, which acts as an air filter for obtaining dust-free air.

The collar P is attached to the end of the piston rod of the air pump by a pin, which slides in vertical slots when the air pump is working. Two oiled silk valves, one on the piston and another above it, V and V′, cause the air to pass from the chamber through the pump in an outward direction only; one of the vertical supports, therefore, the left, is merely a small air pump. The lower part of the collar P works over marks on the brass tube, which is graduated to show both the volume of air introduced into the chamber and also the proportion this bears to the total chamber capacity. The right hand column of the apparatus consists of the threeway stopcock (1), which communicates with the outside air when horizontal, and with the lower measuring stopcocks and the air filter when vertical. When this stop-


C
L
C
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cock is at an angle of 45° the inlet for air is completely closed. The bore of the second stopcock (2) has a capacity of 0·25 cc., and, when horizontal, air with its quota of dust can be aspirated through it by means of the attached piece of flexible tubing. When the stopcock is turned vertical, this measured volume of dusty air enters the partially exhausted chamber in a current of filtered (dust-free) air from the filter F. When a smaller volume of dusty air is required the stopcock No. 3 is used in the same way, No. 2 being kept vertical. The bore of this has a capacity of 0·05 cc., and this is the smallest volume which the instrument is constructed to examine. A small mirror with a dark centre is attached to the filter column, and serves to give a dark ground illumination to the micrometer glass plate.

The principle of the method may be shortly stated as follows: When the air of the chamber is saturated with water vapour and dust is present, a single stroke of the air pump rarefies the air in the chamber. The rarefaction is accompanied by a sudden cooling, brought about by the expansion of the air in the chamber. The cooling causes the air to become super-saturated with water vapour, and the excess condenses on the dust particles as nuclei, causing a fine rain of dew drops to fall on the micrometer as seen through the eye-piece of the instrument. If only filtered air be present, and if the instrument is in adjustment, no dew drops fall when a stroke of the air pump is made. If a small volume of dusty air is introduced, the falling dew drops are few, and if the volume of dusty air is increased a corresponding increase in the number of dew drops occurs. Inside the chamber is a stirrer or fan, movable from the outside, which is stirred from side to side for a short time before making a count in order to mix the dusty air uniformly, with the dust-free air. It is necessary to decide on the volume of dusty air which must be introduced into the chamber to give a countable number of dew drops on one square, by a preliminary experiment. Dr. Aitken recommends that the number of drops falling be not less than one and not more than five on each square, and the volume of air tested must be chosen accordingly. With practice, however, particles

up to ten on a square can be easily counted. With dusty London air in a quiet room we almost invariably found it necessary to use the smallest stopcock, *i.e.*, giving a volume of 0·05 cc.

The following experiments will illustrate the results we obtained with the instrument :—

Following Dr. Aitken's instructions, ten successive counts were made in each experiment and the average taken.

The first series of observations were made during the month of December in a western suburb of London. The open air was found to contain 20,000 dust particles in a cc., whilst on the same day a count made in a quiet room close by gave 44,000 dust particles per cc. The next counts were made in the centre of London. The apparatus was placed on the roof of a house and experiments made at different times, when the air was quiet and when it was windy. The enormous number of dust particles, far exceeding 500,000 per cc., entering the instrument on each occasion, rendered a count perfectly impossible. The millions of dust particles as they entered the chamber reproduced on a small scale a London fog. Nothing could have illustrated more vividly the chronic pollution of the air caused by chimney smoke. We were more successful in the yard of the same house, the average number of dust particles being 300,000, with an occasional rise to 500,000, in a cc. At the same time it should be noted that the number of dust particles entering the instrument varies greatly in the course of the same series of experiments. A puff of wind will suddenly produce an uncountable number, and a repeat is then necessary. The above, however, may be taken as a typical result, viz., 300,000 per cc.

A number of observations were then made in a room of the house, and the following is a representative result. Four successive counts were made, each being the mean of ten.

First count—490,000 per cc.
Second ,, 231,000 ,,
Third ,, 570,000 ,,
Fourth ,, 216,000 ,,

The personal equation undoubtedly produces variations

in the results. For example, working alternately in the same room one of us obtained in two of the above sets of ten observations 216,000 and 231,000, whilst the other obtained 570,000 and 490,000 in a cc. In addition a slight air current, or the movement of some one in the room, may produce a temporary rise in the number of particles in the air in the neighbourhood of the instrument. The air of various rooms was examined in the same way. The number of dust particles found averaged 300,000—400,000 per cc. The dust contamination of the air in the centre of London is about 900 per cent. greater than that of the air in a quiet suburb. When gas is being burnt, or when smoking is going on in a room, the dust particles increase from thousands to millions. A count was made both before and immediately after lighting cigarettes. The number of dust particles at once rose from 360,000 to an estimated amount of two millions in the cc.

We next proceeded to estimate the ratio existing between the number of dust particles and the number of organisms present in a given sample of air. The bacteriological examination was carried out by means of Hesse's tubes and specially constructed glass bulbs. In the case of Hesse's tubes ten litres of air were slowly aspirated over the film of nutrient gelatine with which they were coated. The glass bulbs employed were of a known capacity, viz., 250 cc., and each contained enough gelatine to coat the inside of the glass. After sterilization the bulbs were exhausted of air and hermetically sealed. On being opened a given volume of air entered (250 cc.) along with the organisms it happened to contain. In both instances the number of colonies obtained were counted. These cultivation experiments were, of course, made simultaneously with the dust count. The open air in the centre of London was tested in this fashion. Two experiments yielded respectively 568,000 and 318,000 dust particles in the cc.; ten litres of the same air contained 83 organisms, of which 28 were moulds and 55 bacteria. If we take the lowest result obtained, viz., 318,000 dust particles in a cc., it will be found that there was just *one* organism to every 38,300,000 dust particles present in the air.

An examination was made of the air in a room. Taking the mean of twenty counts, the number of dust particles was 350,000 in a cc. The number of organisms found in ten litres of the same air was nineteen, viz., twelve moulds and seven bacteria; in other words amongst 184,000,000 particles of dust, only *one* organism could be detected. The relative proportion of bacteria to the amount of dust present in air has not, so far as we are aware, been worked out in combination before, and the results serve to present the facts in perhaps a new and striking manner. The figures illustrate forcibly the poverty of the air in bacteria even when very dusty, and also the enormous dilution they undergo. In the daily experience of the laboratory we have a practical illustration of this, the culture tubes of organisms in constant use remaining pure notwithstanding frequent sub-cultivation. And when a contamination occurs, how frequently it is due to imperfect sterilisation or the fingers of the operator. The practical sterility of the air of bacteriological laboratories as regards pathogenic bacteria is also a noteworthy point.

The observations we have recorded are of course capable of extension and amplification in many ways by anyone who may care to work on similar lines.

A few practical points may be noted regarding the working of Dr. Aitken's ingenious apparatus. The first thing that strikes one is the unequal distribution of the particles on the various squares. For example, in one count we obtained 4 and 11, 3 and 5, 2 and 9, 3 and 10, 1 and 5, 2 and 6, particles on adjoining squares. Dr. Aitken acknowledges this difficulty in not getting uniform readings at each test, and furnishes the following explanation. If the air tested is of uniform impurity, the number of particles falling on the same square at each test will frequently vary from 1 to 4. A uniform result cannot be obtained because the distribution of the nuclei is not uniform when the number is small. If a surface be divided into squares and exposed to a slight shower of rain, some of the squares will contain six drops and others only one or two.

A street pavement at the commencement of a shower of

rain shows the same irregularity in the number of drops as is noticed in the dust counter. Differences also occurred between successive estimations by the same observer. For example, in a series of ten observations we obtained the following figures, being the means of counts on two squares —7, 6·5, 3, 7, 6, 11, 5·5, 5·5, 2 and 3·5, *i.e.*, counts varying between 2 and 11. This illustrates the variations in dustiness of different portions of the air in the same room. We have come to the conclusion that the instrument is not one of which much practical use can be made for hygienic purposes. The number of dust particles fluctuates so largely, even in comparatively quiet air, that the observations are only roughly comparable, although made under the most favourable conditions; and in those cases where it would really be of value to make comparative estimations of the amount of dusty impurity in the atmosphere, *e.g.*, factories, crowded dwellings, &c., the number of dust particles is so large that the instrument as at present constructed is unavailable for the purpose. The portable dust counter is only designed for the registration of dust particles up to 500,000 in the cc., and in impure disturbed air the number is much greater. It need hardly be said that these are considerations which in no way affect the great scientific value of the instrument. We also made a few experiments with the koniscope. This instrument was devised by Dr. Aitken for testing very impure air. The relative amount of dusty impurity is estimated by drawing the air into a tube closed at the ends by two glass plates, lined with moistened bibulous paper, and then exhausting by means of an attached air pump. On looking through the column of air in the tube while condensation is taking place, varying tints of colour are seen according to the amount of dust present.

This instrument has to be calibrated by means of simultaneous experiments with the dust counter. Our manipulation of the instrument did not prove very successful, and it is hardly adapted for general use.

We are indebted to Dr. Aitken for much valuable advice. The dust counter employed was obtained from White, of Glasgow.

ON A CONVENIENT METHOD OF PRESERVING LIVING PURE CULTIVATIONS OF WATER-BACTERIA, AND ON THEIR MULTIPLICATION IN STERILISED WATER.

BY JOSEPH LUNT, B.Sc., F.I.C., F.C.S.

THOSE who are engaged in bacteriological laboratories where a large number of different species of bacteria are kept constantly under cultivation, will have experienced the difficulty of preserving their pure cultures true to the original type, and also the disappointment of finding a cultivation quite dead or much degenerated when left for too long a time without re-sowing on fresh media.

Even when frequently transplanted to fresh media many species alter in many of their original characters, which they possessed when freshly isolated from their natural habitat and environment. Some lose liquefying power, others their pigment-producing properties, and many lose the characteristic mode of forming colonies on gelatine.

Bacteriologists have become so accustomed to transplanting their cultures on the various albuminous media, which in the early days of the subject were found so advantageous for the growth of pathogenic bacteria, that they have almost come to be regarded as essential for their cultivation.

To the many who now devote their special attention to the large group of water-bacteria, the following method of cultivation in sterilised tap-water may prove advantageous in the following respects:—

(1) It obviates the necessity of frequently transplanting cultures to fresh media, not to mention the saving of labour in frequently preparing fresh media for their reception. It

is found that such water-cultures remain in good condition over very long periods, during which they would have died on the ordinary media.

(2) The cultures are preserved in their natural habitat, and on re-culture in gelatine plates and on various other media grow vigorously with their original characters which were observed when freshly isolated from water.

This is of much importance, for where such cultures are preserved for purposes of comparison, there is not the same degeneration and modification of the characters of cultures which are so often found to have taken place in those which are carried on from tube to tube for long periods on the ordinary media.

(3) The method serves to differentiate those organisms which may properly be classed together as water-bacteria, which find in water all their requirements in the shape of nutritive materials, and in which medium they will thrive for very long periods — if not indefinitely — from those organisms which in most cases may be found to lie outside the group of water-bacteria proper, and which will not thrive under these conditions.

The experiments have been made with the following organisms, viz. :—

B. fluorescens liquefaciens.
B. fluorescens fuscus (new species).
B. violaceus.
B. nubilus.
B. iridescens.
B. prodigiosus.
B. giganticus (new species).
B. coli communis.

Bacillus Fluorescens Liquefaciens.

A single platinum loop-ful of the surface growth on agar was suspended and evenly distributed by shaking in 25 cc. of sterile distilled water (the middle portion of the distillate from alkaline permanganate) in a small Chamberland flask. No nutritive material was transferred by the needle except the bodies of the bacilli themselves; 2 cc. of this liquid containing the bacteria in suspension was transferred to

each of three other similar flasks holding 25 cc. of sterile (1) distilled water (as above); (2) London tap-water; (3) London sewage.

These were examined from time to time to ascertain whether the bacteria were still alive, and after two years a single platinum loop-ful of the liquid in each case gave very numerous colonies of the organism introduced, when smeared on the surface of agar.

Four months (131 days) after the above first sowing, a single platinum loop-ful of the above sown tap-water was introduced into another flask containing a litre of sterilised tap-water. This flask, after a further thirteen months contained the living bacilli in large numbers, for a single platinum loop-ful of the water gave a crowded culture when smeared on the surface of agar.

In another experiment the original 25 cc. flask of London tap-water sown with this organism was again tested when twelve months old by taking a single platinum loop-ful of the water and sowing it in a litre of sterile London tap-water. This water culture also was crowded with the living bacilli when examined six months later.

In the case of B. fluorescens liquefaciens then, we have vigorous living cultures which have been kept in tap-water for (1) six months; (2) thirteen months; (3) two years. At the end of these periods the liquefying power of the organism had been fully maintained.[1] Moreover, a flask containing only pure distilled water also contained large numbers of the living bacilli after two years.

From the above water-culture nearly 14 months old, a count was made of the number of living bacilli it contained in a cubic centimetre and it was found to contain 6,800 bacilli per cc.

[0·5 cc. was added to 50 cc. of sterile water and well mixed, from this 0·5 cc. sown in each of two plates, which yielded 33 colonies in one case and 35 in the other.]

That is, 6,800,000 bacilli still survived in the litre of

[1] Roscoe and Lunt *Phil.* (*Trans. Royal Society*, vol. 182, B, plate 41) show that this organism loses liquefying power in a marked manner when grown in the ordinary way from culture to culture.

water-culture 14 months old, which had originally been sown with a single loop of a previous 4 months old water-culture.

In order to demonstrate the enormous multiplication of B. fluorescens liquefaciens in tap-water, 0·5 cc. of the 100 times diluted water-culture which contained 34 bacilli, as shown by the above plate cultures, was sown in 750 cc. of sterile London tap-water, β.

After three days' incubation at room temperature, the water-culture β. was found by plate culture to contain 6,000 bacilli per cc. therefore, in 3 days the 34 bacilli sown had multiplied to 4,500,000.

The small 50 cc. flask (α) used for the above dilution was also examined after three days, and it was found to contain 725,000 bacilli per cc., and the 3,400 originally sown had increased to 36,250,000.

These figures show the enormous capacity for supporting bacterial life possessed by comparatively pure drinking water.

The water-culture β was further examined when 6 days and 21 days old, and the numbers showed a still further large increase, being 460,000 per cc. on the 6th day and 550,000 per cc. on the 21st day.

The above results are shown more clearly in Table I., which gives further numerical details of the examination of the above water-cultures.

TABLE I.

Water-cultures of Bacillus Fluorescens Liquefaciens.

Flask Number.	Vol. of Water in Flask.	Vol. of Water-culture Sown.	Age of Water-culture used for Sowing.	Total Bacilli Sown.	Duration of Incubation.	Increase per Bacillus.	Bacilli[1] per cc.	Total Number of Bacilli in Flask.
α	50 cc.	0·5 cc.	14 mos.	3,400	3 days	10,661	725,000	36,250,000
α	50 cc.	0·5 cc.	,,	3,400	6 days	8,529	580,000	29,000,000
β	750 cc.	0·005 cc.	,,	34	3 days	132,353	6,000	4,500,000
β	750 cc.	0·005 cc.	,,	34	6 days	10,147,058	460,000	345,000,000
β	750 cc.	0·005 cc.	,,	34	21 days	12,132,353	550,000	412,500,000
β	750 cc.	0·005 cc.	,,	34	85 days	11,029,411	500,000	375,000,000

[1] These countings were made with the diluted water-cultures, *e.g.*, the first count from flask α gave 145 colonies on a plate sown with 0·1 cc. taken from a 50 cc. flask which had been sown with 0·1 cc. of the water-culture α; therefore the water-culture α contained 725,000 bacilli per cc.

It will be noticed that in the small flask (α) the actual increase per bacillus is much smaller, being less than one-tenth, than in the large flask β. In the small flask with its larger initial sowing (100 times) the effects of over-crowding are well seen, and whereas this flask shows a marked decline in numbers on the 6th day the larger flask shows a marked further increase which is still further augmented on the 21st day, though it is probable that a maximum may have occurred between the two observations.

The numbers showing "bacilli per cc." show very clearly the comparative over-crowding in the small flask on the 3rd day.

The column "Increase per bacillus" also shows the effect of this in the marked diminution in the rate of multiplication between the 3rd and 6th day in the large flask as compared with the rapid multiplication on the first three days.

As regards the source of nutriment which sustains such large numbers of bacteria, it should be remembered that in addition to the natural saline constituents of the water a trace of the materials of the glass vessel is probably dissolved during the sterilisation of the water, and in addition to the natural traces of organic matter in the water a further absorbtion of organic matter from the air by the water may be assumed to take place.

These slight causes may be sufficient to account for the fact of the marked longevity of the above-named water-cultures sown in the pure distilled water obtained from the middle portion of the distillate from alkaline potassium permanganate.

The degenerated and dead bacilli in their decay also set free nutriment of the very kind required, and a constant cycle of changes may be supposed to take place whereby such material is assimilated by new generations of bacilli, which succeed the older ones almost as fast as they degenerate and decay. Ultimately an equilibrium is established between the rate of decay and the rapidity of multiplication, and it is quite conceivable that such a state of things may go on indefinitely and thus account for the remarkable longevity of such water-cultures.

Then again, the bacilli have full possession of the field for the growth of their own species, and there is no antagonism such as occurs in natural water, owing to the presence of many other species.

Water-cultures of Bacillus Fluorescens Fuscus.

A small particle of the surface growth from a twenty-four hours' old agar culture was sown into 500 cc. of sterilised London tap-water (1), and after eight months, loop smears on agar gave crowded colonies.

On counting by plate cultivations this eight months old water-culture was found to contain 1,000,000 bacilli per cubic centimetre.

A second water-culture (2) was made from flask (1) on the same day that the plates were made, using $\frac{1}{5000}$th cc. or 200 bacilli for the sowing. After three days these became 90,000,000.

In a smaller flask (50 cc. *a*) used for dilution purposes in the above sowing, with an initial sowing 500 times greater than in the large flask (= 100,000 bacilli) the increase was only to 9,125,000, again showing the effects of overcrowding very clearly.

The results of these and other experiments with this organism are given in Table II.

TABLE II.

Water-cultures of Bacillus Fluorescens Fuscus.

Flask Number.	Vol. of Water in Flask.	Vol. of Water-culture sown.	Age of Water-culture used for Sowing.	Total Bacilli sown.	Duration of Incubation.	Increase per Bacillus.	Bacilli per cc.	Total Number of Bacilli in Flask.
a	50 cc.	0·1 cc.	8 mos.	100,000	3 days	91	182,500	9,125,000
2	750 cc.	0·0002 cc.	8 ,,	200	3 ,,	450,000	120,000	90,000,000
3	750 cc.	0·0002 cc.	3 days	24	5 ,,	17,812,500	570,000[1]	427,500,000
3	750 cc.	0·0002 cc.	3 ,,	24	10 ,,	23,437,500	750,000[1]	562,500,000
4	750 cc.	0·0002 cc.	10 ,,	150	7 ,,	1,300,000	260,000	195,000,000[2]

[1] Plates sown with 0·1 cc. taken from a 50 cc. flask which had been sown with 0·1 cc. from the cultures under examination yielded 114 and 150 colonies respectively.

[2] The reason for the lower rate of multiplication in this last experiment was not ascertained.

The name B. fluorescens fuscus is one which I have given to an organism isolated from filtered water, and which is believed to be a new species. It closely resembles B. fluorescens liquefaciens in plate culture, and young colonies might easily be mistaken for the latter, as they exhibit a slight green fluorescence. Older colonies and the completely liquefied plates, however, show an intense brown coloration of the liquefied gelatine. The bacilli are very motile, and on potato a deep yellowish brown thick moist growth appears. On agar also this brown coloration is very marked, and the colouring matter diffuses itself throughout the agar. The organism grows well in broth at 37°, and forms a thin rust-red film. In young sloped gelatine cultures the green fluorescence is well marked. A full description of the organism will shortly be published.

Water-cultures of Bacillus Violaceus.

This organism shows the same remarkable longevity in water-cultures as does B. fluorescens liquefaciens.

Cultures were made in (1) distilled water; (2) London tap-water; and (3) London sewage exactly in the same way as with B. fluorescens liquefaciens, and after twenty-three months vigorous cultures were obtained from the flasks on sowing on various other media, a single loop of the liquid yielding numerous colonies in each case.

From the first tap-water culture (α) 108 days old, a single loop-ful was taken, and this was sown in 1,000 cc. of sterilised tap-water. When this latter (β) was fourteen months old $\frac{1}{200}$ cc. was sown into 750 cc. of sterilised tap-water, and of this latter (1) when fifteen days old $\frac{1}{5000}$ cc. was sown into another 750 cc. flask (2). The sowing was found to be 195 bacilli, and after six days these had increased to 506,250,000. From this again another flask was sown (3), and the sowing of 135 bacilli resulted in an increase in six days to 382,500,000.

Further particulars of these experiments are given in the following table.

TABLE III.

Water-cultures of Bacillus Violaceus.

Flask Number.	Vol. of Water in Flask.	Vol. of Water-culture Sown.	Age of Water-culture used for Sowing.	Total Bacilli Sown.	Duration of Incubation.	Increase per Bacillus.	Bacilli per cc.	Total Number of Bacilli in Flask.
β	1,000 cc.	Loop	108 days	?	14 mos.	..	..	..
1	750 cc.	0·005 cc.	14 mos.	?	15 days	..	975,000	731,250,000
2	750 cc.	0·0002 cc.	15 days	195	6 ,,	2,596,153	675,000[1]	506,250,000
3	750 cc.	0·0002 cc.	6 ,,	135	6 ,,	2,830,000	510,000	382,500,000
3	750 cc.	0·0002 cc.	6 ,,	135	64 ,,	..	450,000	337,500,000

[1] It will be seen that this flask when first sown only contained approximately 1 organism in every 4 cc., and in six days the numbers rose to 675,000 organisms in every cubic centimetre.

Bacillus Nubilus.

Water-cultures of bacillus nubilus were also examined in the same way: $\frac{1}{200}$ cc. of a water-culture more than 7 months old was sown into 1000 cc. of sterilised tap-water, and after 15 days $\frac{1}{5000}$ cc. of this latter was sown in 750 cc. of water. The sowing was found to be 122 bacilli [showing the flask from which the culture was taken to contain 610,000 bacilli per cc. or 610,000,000 bacilli resulted in 15 days from a sowing of $\frac{1}{200}$ cc. of a 7 months' old water-culture], the 122 bacilli became in 6 days 5,250,000 or 7,000 per cc. whereas, when first sown there was only 1 bacillus in every 6 cc.

This rate of multiplication is much slower than in the previous examples, but, the fact of finding 610,000 per cc. in a 15 days old culture shows that this organism thrives very well in sterilised London tap-water.

Bacillus Iridescens

Also remained alive and in good condition in water-culture for 11 months.

Bacillus Prodigiosus.

Bacillus prodigiosus does not exhibit the same rapid rate of multiplication which is shown by the other organisms experimented with. Nevertheless, it survived for 10 months in

water-cultures, and certainly many of the surviving individuals show an unimpaired and typical formation of pigment and the liquefying power is fully maintained.

A small particle of a surface growth on potato, 3 days old, was sown in 500 cc. of sterilised tap-water.

After 7 months, loop smears on agar gave from 5 to 7 colonies, but only about $\frac{1}{7}$th of these were well-pigmented colonies, the rest being white colonies, which only developed red spots at their edges later.

The same remarks as to pigmentation hold good for gelatine plates. Nevertheless, those colonies which produced pigment, either on agar or plates gave very typical and brilliant cultures when further cultivated on potato or agar. As it is uncertain whether the water was in the first instance sown with a mixture of the white and coloured varieties, or from a potato culture grown from a well-pigmented isolated colony, this experiment does not decisively prove the loss of power of forming pigment whilst growing in the water, since the colourless varieties, which may have been present, may have multiplied more extensively than the coloured variety. Certainly, after $9\frac{1}{2}$ months a coloured and a colourless race were growing side by side in the water-culture. 1000 cc. of sterilised tap-water was sown with 3 loops from the above culture, corresponding to a sowing of 21 bacilli, of which we may say 3 were of the coloured race. After three days loop smears on agar and sowings on gelatine plates, corresponding to $\frac{1}{200}$, $\frac{1}{1000}$, and $\frac{1}{5000}$ cc. remained sterile, showing that the numbers present were very small.

The flask sown with 0·5 cc. also remained sterile, showing that 0·5 cc. contained no organisms.

Water-cultures of Bacillus Giganticus.

Cultures of this organism were made in exactly the same way as in the case of B. fluorescens liquefaciens and B. violaceus in (1) distilled water; (2) London tap-water; (3) London sewage, and after twenty-three months each flask gave vigorous cultures of the organism when a loop-ful of the water was smeared on the surface of agar.

From the first tap-water culture, when over three months

old, a loop-ful was sown in 1,000 cc. of sterilised tap-water. This culture also after sixteen months gave numerous vigorous colonies on agar from loop smears.

When the above culture was nearly fourteen months old $\frac{1}{200}$ cc. was sown in 750 cc. of sterilised London tap-water, and after sixteen days a count was made and it was found to contain 950,000 bacilli per cc. or 712,500,000 in the 750 cc.

From this sixteen days old culture $\frac{1}{1250}$ cc., corresponding to a sowing of 760 bacilli was added to another flask containing 750 cc. of water. In six days this was examined by plate culture and it was found to contain 800,000 bacilli per cc. and the 760 bacilli had therefore multiplied to 600,000,000. The following table shows the results obtained with B. nubilus and B. giganticus.

TABLE IV.

Water-cultures of Bacillus Nubilus and Bacillus Giganticus.

Flask Number.	Vol. of Water in Flask.	Vol. of Water-culture Sown.	Age of Water-culture used for Sowing.	Total Bacilli Sown.	Duration of Incubation.	Increase per Bacillus.	Bacilli per cc.	Total Number of Bacilli in Flask.
Bacillus Nubilus.								
1	1,000 cc.	$\frac{1}{200}$ cc.	7 mos.	?	15 days	?	610,000	610,000,000
2	750 cc.	$\frac{1}{5000}$ cc.	15 days	122	6 ,,	43,032	7,000	5,250,000
Bacillus Giganticus.								
1	750 cc.	$\frac{1}{200}$ cc	14 mos.	?	16 ,,	?	950,000	712,500,000
2	750 cc.	$\frac{1}{1250}$ cc.	16 days	760	6 ,,	786,842	800,000	600,000,000

Water-cultures of Bacillus Coli Communis.

A small particle of a three days old gelatine culture of B. coli communis, recently isolated from fæces and proved to be normal in character, was stirred into 750 cc. of sterilised tap-water.

After five days[1] the number present was ascertained by

[1] After seven days more, *i.e.*, when twelve days old, the numbers present remained practically the same within the limits of experimental error, $\frac{1}{1250}$ cc. gave 72 colonies.

After thirty days more, *i.e.*, when 35 days old, $\frac{1}{1250}$ cc. gave 53 colonies.

plate culture $\frac{1}{1250}$ cc. gave 62 colonies, therefore 77,500 bacilli per cc. were present.

$\frac{1}{1250}$ cc. was at the same time sown into another flask containing 750 cc. of sterilised tap-water, equivalent to sowing 62 bacilli. This initial sowing corresponded to a sowing of one bacillus in every 12·1 cc.

After three days' incubation at room temperature this water was again examined by plate culture, and it was found that 6·6 cc. gave only one colony, which happened to appear in one out of the six plates sown with 0·3 cc. The others containing 3 cc., 2 cc., 1 cc., 0·2 cc. 0·1 cc., all remained sterile. This shows that practically no multiplication had taken place, because if every bacillus had multiplied into only two, one should have been present in 6·05 cc. Only one bacillus was found in 6·6 cc. and this is quite compatible with the next 5·5 cc. being sterile, thus showing only the same number of bacilli as were originally sown, viz., 1 bacillus in 12·1 cc.

When this water culture was thirty days old it was again examined, and 12 cc. in four plates of 3 cc. each gave only one colony, the other three plates were quite sterile, showing that the numbers present were still identically as at first, viz., 1 bacillus in 12 cc.

The 50 cc. flask used for dilution in the above sowing, and in which, when first sown, 0·2 cc. contained 62 bacilli, was further examined when seven days old, and it was found that 0·2 cc. gave precisely the same number of colonies as at first, viz., 62 showing that the numbers had remained stationary, and being a further confirmation of the first experiment.[1]

Summary and Conclusions.

(1) Certain water bacteria are able to live for very long periods in sterilised water (two years at least).

(2) They are present in the water in very large numbers at the end of that period.

[1] After over five months (161 days) the numbers present had materially decreased, only 31,250 per cc. being present. Six colonies were further examined in various media, and it was found that the cultures obtained from this five months old water had remained normal in character, no diminution in indol production or milk-curdling power or in fermentative action could be detected.

(3) They retain their characters much better in water cultures than when transplanted from time to time on ordinary media.

(4) The paper gives the results of exact quantitative experiments as to the capacity for multiplication in sterilised water possessed by the species examined, which results may be used for comparative experiments with other organisms.

(5) The results give, the author believes for the first time, a reliable basis for the classification of certain species of bacteria in a group called water-bacteria having the following characters:—(*a*) To be found in natural water; (*b*) capable of living for very long periods in sterilised water; (*c*) capable of very rapid multiplication in sterilised water; (*d*) showing no signs of degeneration when kept for long periods in sterilised water.

(6) The method of preserving living pure cultures of water-bacteria in water-cultures involves far less labour, and gives much better and more certain results than the methods usually employed.

(7) Species which do not belong to the water-bacteria group do not behave in the same manner when sown in water-cultures as do water-bacteria.

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